J Obstet Gynecol Ind Vol. 53, No. 4 : July/August 2003 Pg 398-400

Cytogenetic and Molecular Study in a 46, XY Woman

F J VinSheth[1], J J Sheth[1], V R Hyderabadi[1], K McElreavey[2], C Krausz[2]

[1]Foundation for Research in Genetics and Endocrinology (FRIG), 20/1, Genetics Centre, Bimanagar, Satellite Road, Ahmedabad - 380 015, India. [2]Unite d'Immunogenetique Humaine, Institute Pasteur, 25 rue du Dr Roux, 75724, Paris Cedex 15, France

Introduction

The testis determining gene, SRY (sex determination region, Y) is located on the short arm of the Y chromosome and sex determination in human depends upon the action of SRY gene[1]. Mutations in the SRY gene can cause a failure of testicular development leading to 46, XY complete goandal dysgenesis (Swyer Syndrome). These individuals are characterized by a female phenotype, normal to tall stature, bilateral dysgenetic gonads, sexual infantilism with primary amenorrhoea, eunuchoid habitus and 46, XY karyotype. Approximately 25% of 46, XY women with gonadal dysgenesis carry mutations in the SRY gene[2]. In other rare cases duplications of a region termed "Dosage Sensitive Sex reversal" (DSS) have been observed with male to female sex reversal and 46, XY karyotype [2,3]. The molecular basis of the remaining cases is unknown, although a locus at distal chromosome 9p has been associated with this phenotype[3]. In most cases of 46, XY gonadal gysgenesis, the gonads are removed, as these individuals are at high risk to develop gonadoblastomas[4,5]. Gonadoblastoma is a highly malignant testicular tumor defined histologically by the presence of germ cells and cells derived from the sex cords (granulose cells and Sertoli cells). Related germ cell tumors are the endodermal sinus tumors (EST ; otherwise known as yolk sac tumors or yolk sac carcinomas) that usually involve the gonads and extragonadal region. The etiology of endodermal sinus tumors is unknown. Abdominal and/or pelvic pain is the most frequent presenting symptom.

Here we describe a rare case of a 46, XY woman who had a peritoneal endodermal sinus tumor. Analysis of the SRY gene and the DSS region failed to detect any anomaly that could explain the sex reversal. In addition, the molecular analysis revealed a grossly intact Y chromosome including the region associated with gonadoblastoma. The possible molecular causes of gonadal dysgenesis and tumorogenesis in this patient are discussed.

Paper received on 27/11/02 ; accepted on 25/3/03

Correspondence :
F.J. VinSheth
Foundation for Research in Genetics and Endocrinology
20/1, Genetics Centre, Bimanagar, Satellite Road,
Ahmedabad - 380 015.

Case History

Clinical Details

A 19 year old girl complaining of primary amenorrhoea was first referred for laboratory investigations in August 1995. She was the eldest child born to nonconsanguineous parents. Proband had two sisters and a brother. She was well built with a height of 165cm and a weight of 65 kgs. On clinical investigations she had normal secondary sexual characteristics with masculine features. External genitalia were those of a normal female. Neither scrotal rugosity nor gonads were palpable. Axillary and pubic hair were present. She had been brought up as a female and had female psychosexual orientation.

Investigations

Pelvic sonography at 19 years showed a small infantile uterus with follicular cyst measuring 3.1 x 2.4 cm. in the right gonad while left gonad was not visualized. Endocrine investigations were carried out for circulating androgen concentration. Plasma testosterone was 1.5 ng/ml (NR : 0.2-0.9 ng/ml); free testosterone 2.1 pg/ml (NR: 1.0-3.5 pg/ml), and DHEAS 5400 ng/ml (NR : 1311-2800 ng/ml). Considering her hyperandrogenic status she was given dexamethazone (0.5 mg) at bed time for four weeks. On repeat investigation, plasma DHEAS had dropped to 3000 ng/ml. As she did not menstruate without cyclincal hormonal preparation, she was investigated for serum FSH, LH and Prolactin level which were 95.0 mlu/ml (NR : 4.5-20 mlu/ml), 27.0 mlu/ml (NR: 5-20mlu/ml) and 44.0 ng/ml (NR : 3-15 ng/ml) respectively. Thyroid function tests (T_3, T_4 and TSH (IRMA)) were normal at this time. These findings were suggestive of primary ovarian insufficiency with hyperandrogenism, together with masculine features. Her chromosomal constitution was determined by a stimulated blood lymphocyte culture using standard G- and C- banding techniques and revealed 46, XY karyotype in all cells (Photograph 1). Based on these observations, the proband was considered to have either gonadal dysgenesis or true hermaphroditism. She was advised to have laparoscopy followed by the removal of gonads but she refused it. In April 1997, she complained of acute abdominal pain and had to be taken for an emergency operation. On opening the abdomen, 1.5 liters of blood was found in the peritoneal cavity surrounding a large tumorous mass, which was excised

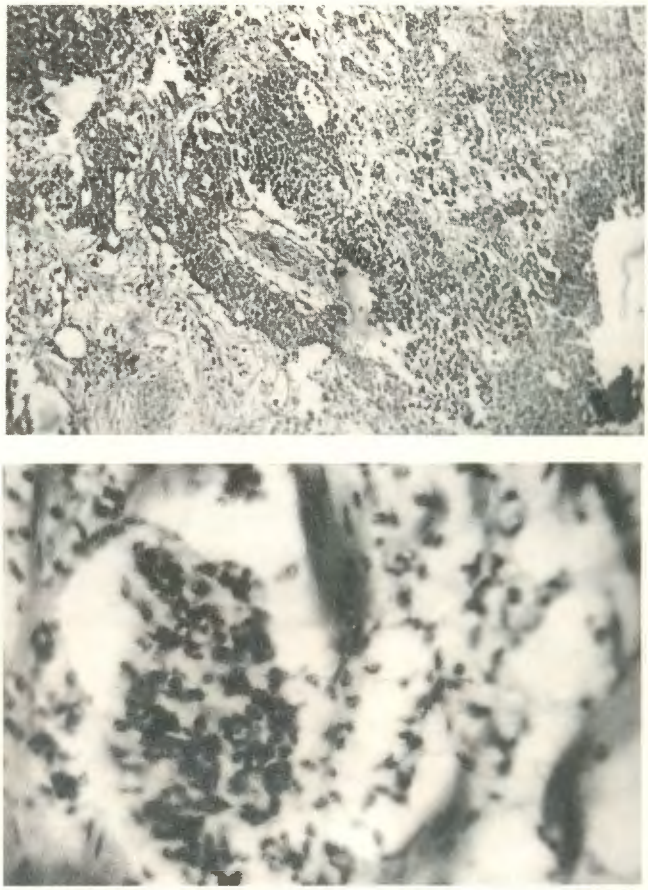
Photograph 1 : Karyotype shows 46,XY chromosomal complement.

leaving both gonads intact. Her serum αFP level at this time was 600 ng/ml (NR: upto 15.0 ng/ml) and βhCG was 8.9 mlu/ml. This has suggested an embryonic malignancy and histopathology of the tumor mass revealed an endodermal sinus tumor (Photograph 2).

In July 1997, a repeat laparotomy was performed and the gonads were removed. On gross examination the specimen was of fallopian tubes with attached ovary and adipose tissue. Histopathological examination revealed dysgenetic gonads. The right gonad was small (3.0cm x 2.0 cm). Ovarian fibrous stroma was observed with calcified cystic walls. A few primitive tubules surrounded by fibrous stroma were observed. The cystic walls were composed of tall columnar epithelium with basal nuclei and mucinous cyst adenoma. The left gonad had a similar structure with some areas of ovarian stroma replaced by a multiple foreign body granulomata.

Molecular Studies

DNA was prepared from peripheral blood lymphocytes and the entire SRY open reading frame was amplified using the primers SRY-alpha (5'-ATGCAATCATATGCTTCTGCTATGT-3') and SRY-omega (5'TTTGTAGCCAATGTTACCCGATT-3'). The amplification conditions were 94°C for initial denaturation (5 min) followed by 35 cycles of 96°C (1 min), 55°C (1 min), 72°C (1 min) and then 72°C for 10 min. The PCR products were purified from a preparative agarose gel (GeneClean II : Bio 101, Inc). The sequenase kit (USB) or the Dye terminator Sequencing Kit (Perkin Elmer) were used to sequence following the instructions of the manufacturers. Southern blotting was performed on StuI digested DNA using the plasmid pY53.3 (which contains the SRY gene)[6] as a probe and the plasmid QST59 which maps within the DSS critical region (at position DXS 319 (Xp21.3). Amplification of Y chromosome sequences was performed using the primer pairs sY70, sY72, sY74, sY84, sY104, sY119, sY123, sY125, sY134, sY152, sY158 and sY254.



Photograph 2 : Tumor cells showing solid undifferentiated areas on the right side and loose meshwork seen in tumor tissue with tuff like tumor cells on the left. (Above - lower power, below - higher power).

Molecular Biology Results

The sequence of the SRY gene was found to be identical to that of a normal 46, XY male. In addition, Southern blotting failed to identify any rearrangement involving the gene (data not shown). Southern blots of the DSS region using the plasmid QST59 as a probe were identical to a normal male control, indicating that the DSS region was present as a single copy. PCR analysis using markers located along the length of the Y chromosome were all positive indicating that the Y chromosome was grossly intact.

Discussion

Genetically, complete 46, XY gondadal dysgenesis is a very heterogeneous disorder with both Y – linked and non-Y-linked forms. Eighty percent of patients with sporadic or familial 46, XY gonadal dysgenesis do not have a mutation or deletion of the SRY gene. The only available report from India has shown the presence of point mutation in SRY region in one of four women with 46, XY genotypes[7]. This indicates that other autosomal or X-linked genes have a role in sex determination[5].

In our case, mutations in the SRY gene or in the DSS locus on the X chromosome were not detected. It is possible that the proband might have harbored mutations in an autosomal sex-determining region, as several cases of gonadal dysgenesis have been described associated with microdeletions of chromosome 9p24.3 [4,8,9].

The proposit described here presented with peritoneal endodermal sinus tumor. This accounts for about one third of the malignant germ cell tumors of the ovary in 46,XY women. The molecular cause of malignancy associated with 46,XY gonadal dysgenesis is yet not clear. Tsuchiya et al.[10] have defined a pericentric region of the Y chromosome which when present in an individual with gonadal dysgenesis is associated with tumorogenesis. Endodermal sinus tumor has been reported in several cases of 46,XY gonadal dysgenesis or Swyer syndrome [5]. Morsy et al[11] described a 27 year old woman (46,XY women) who presented with abdominal pain due to hemoperitoneum from a ruptured abdominal mass. Histopathological study revealed an endodermal sinus tumor associated with gonadoblastoma and dysgerminoma. In another case, a 19-year-old woman was described with a 46,XY genotype and complete gonadal dysgenesis was associated with gonadoblastoma (with dysgerminoma differentiation in both gonads), mature teratoma, embryonic carcinoma and EST [2]. EST. has also been associated with the deletions of chromosome 1[12]. The same molecular mechanism responsible for gonadoblastoma may also result in EST in 46,XY women.

A small critical region on the short arm near the centromere of Y chromosome has recently been proposed as a candidate for gonadoblastoma[10]. Human TSPY (testis-specific protein, Y- encoded) gene family lies within the minimum critical region in Yp[1,3]. The number of copies of TSPY varies between 20-40 in humans. In adult testis, TSPY is expressed in spermatogonia and primary spermatocytes suggesting that it plays a role in spermatogenesis. TSPY is also expressed in testicular tumors (mature seminoma, carcinoma in-situ), in dysgenetic gonads of 46,XY women and in gonadoblastoma[13,15]. Since TSPY also shares sequence identity to the putative proto oncogene (SET), it may also play a role in tumorogenesis[14].

We conclude from the present case study that the detailed investigations of young women with primary amenorrhoea are necessary not only for better management but also for the possible prevention of tumorogenesis.

References

1. Arnemann J, Epplen JT, Cooke HJ et al. A novel Y–chromosomal DNA sequence expressed in testicular tissue. *Nucl Acids Res 1987; 16: 8713–24.*
2. Bremer GL, Land JA, Tiebosch A et al. Five different histologial substypes of germ cell malignancies in an XY female. *Gynecologic Oncology 1993; 50: 247–8.*
3. Manz E, Schnieders F., Muller Brechlin A et al. TSPY–related sequences represent a microgeterogeneous gene family organized as constitutive elements in DYZ5 tandem repeat units on human Y chromosome. *Genomics 1993; 17: 726–731.*
4. McElreavey K, Fellous M. Sex determination and the Y chromosome. *Am J Med Genet 1999; 89: 176–85.*
5. Kyriakie S, Harry O. Familial sex reversal : A review. *J Clin Endocri Metab 2000; 85 : 483–93.*
6. Sinclair AH, Berta P, Palmer MS et al. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA–binding motif. *Nature 1990; 346: 246–54.*
7. Kucheria K, Mohapatra I, Ammini AC et al. Clinical and DNA studies on 46, XY females with gonadal dysgenesis. A report of six cases. *J. Reprod Med 1996; 41 : 163–6.*
8. Veitia RA, Nunes M, Quitanamurci L et al. Swyer-syndrome and 46,XY partial gonadal dysgenesis associated with 9p deletions in the absence of monosomy 9 p syndrome. *Am J Hum Genet 1998; 63: 901–5.*
9. Verp MS, Simpson JI. Abnormal sexual differentiation and neoplasia. *Cancer Genet Cytogenet 1987; 25: 191 – 218.*
10. Tsuchiya K, Reijo R, Page D. Gonadoblasoma : Molecular definition of the susceptibility region of the Y chromosome. *Am J Hum Genet 1995; 57: 1400–7.*
11. Morsey AH, al-Fadly A, Mokhtar S et al. Swyer syndrome : an unusual presentation. *Int J Gyn Obstet 1995; 49: 185–6.*
12. Perlman EJ, Valentine MB, Griffin CA. Deletion of 1p36 in childhood endodermal sinus tumors by two-color fluorescence in situ hybridisation : a pediatric oncology group study. *Genes, Chromosomes and Cancer 1996; 16: 15–20.*
13. Lau Y, Chou P, lezzoni J. Expression of a candidate gene for the gonadoblastoma locus in gonadoblastoma and testicular seminoma. *Cytogenetic Cell Genet 2000; 91: 160–4.*
14. Van Lindern M, Van Baal S, Wiegant J et al. Can, a putative oncogene associated with myeloid leukemogenesis, may be activated by fusion of its 3' half to different genes : characterization of the SET gene. *Mol Cell Biol 1992; 12: 3346–55.*
15. Schniders F, Dork T, Arnemann J et al. Testis–specific protein Y encloded (TSPY) expression in testicular tissues. *Hom Mol Biol 1996; 5 : 1801–7.*