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Short communication

C601S mutation in the androgen receptor results in partial loss of androgen function☆

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ABSTRACT

The present study was undertaken on a case of partial androgen insensitivity syndrome to look at the etiology of the disorder. The patient exhibited a female phenotype despite 46,XY chromosome complement. Direct DNA sequencing of coding region of the androgen receptor gene in this case revealed a 2329G>C substitution (cDNA sequence reference) in exon 3 of the gene. The substitution resulted in replacement of Cys with Ser at codon 601 of the ligand-binding domain of the protein. Analyses on 200 control samples revealed absence of this substitution(s). *In vitro* assays were done using COS-1 cells. The mutation resulted in partial (~40%) loss of ligand-binding and significant (~70%) loss of downstream transactivation function. The mutation was absent in the controls. The findings are particularly interesting since another substitution at the same codon (TGC–TTC) has been reported in association with complete androgen insensitivity syndrome.

1. Introduction

Androgens (testosterone and dihydrotestosterone) initiate the process of secondary sexual differentiation during prenatal stage. The message is conveyed through androgen receptor (AR), such that receptor–testosterone complex signals the differentiation of Wolffian duct during embryonic life, regulation of secretion of leutinizing hormone by hypothalamic–pituitary axis and spermatogenesis, and receptor–dihydrotestosterone complex promotes the development of external genitalia and prostate during embryogenesis and is also responsible for changes, which occur at puberty in males [1]. Androgen receptor is encoded by the *AR* gene mapped onto the long arm (Xq11-12) of X-chromosome [2]. Eight exons of the gene encode a protein with 919 amino acid residues. A member of nuclear receptor super-family, AR protein has a domain organization consisting of N-terminal domain (NTD), DNA binding domain (DBD) and ligand-binding domain (LBD). In addition to ligand binding, LBD is also involved in nuclear localization, receptor dimerization and interaction with other proteins [3].

The end organ resistance to androgens is named as androgen insensitivity syndrome (AIS) (MIM# 300068). AIS is a very rare disorder with a frequency of approximately 1 in 30,000 live births [4]. Mutations in the *AR* gene are the most common cause of AIS. The phenotype in androgen insensitivity depends on the extent of loss of androgen function. Individuals with complete androgen insensitivity syndrome (CAIS) have female external genitalia, usually with small labial folds, a short blind ending vagina, absent Wolffian duct derived structures and prostate, gynecomastia, scanty pubic and axillary hair [5]. In partial androgen insensitivity syndrome (PAIS), several different phenotypes are evident, with predominantly female phenotype (female external genitalia, pubic hairs with or without clitoromegaly and partially to completely fused labia) in most severe form, ambiguous genitalia to predominantly male phenotype with micropenis, perineal hypospadias and cryptorchidism in less severe forms [6]. The later group of patients is also termed as Reifenstein syndrome (MIM# 312300). PAIS patients are assigned a grade according to the severity of androgen insensitivity and affinity of the phenotype with male or female pattern. Individuals with mildest form of androgen insensitivity (MAIS) usually have normal male genitals and internal male structures, and during puberty may have breast enlargement, sparse facial and body hair, and small penis [7]. Some affected individuals who otherwise are normal male may also have impaired sperm production resulting in oligozoospermia or azoospermia [8].

So far, more than 500 mutations have been reported in the *AR* gene in various grades of AIS [9]. A substitution at codon 601 (TGC–TTC) has been reported in a case of complete androgen insen-

☆ S.R., N.J.G., L.S. and K.T. have nothing to disclose.

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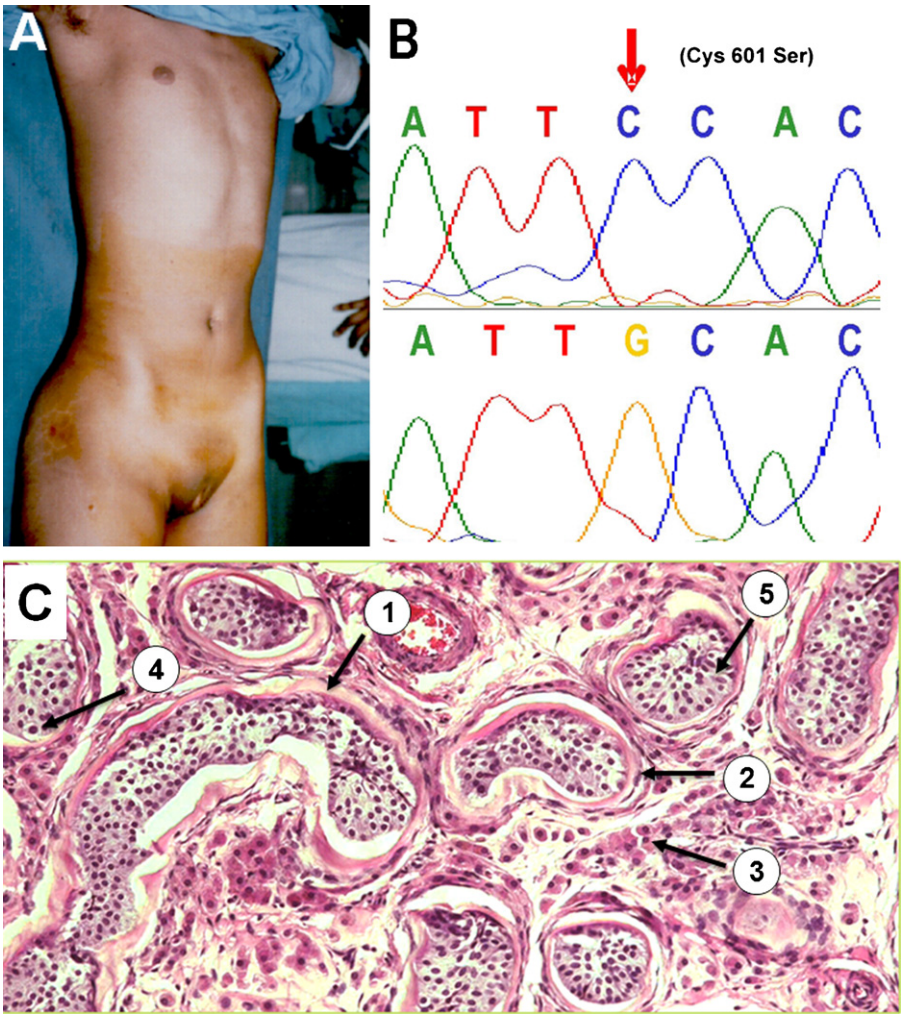

Fig. 1. Phenotypic, histological and genetic evaluation of the patient. (A) Phenotypic features showing ambiguous genitalia. (B) Electropherogram showing the mutation in *AR* gene. The electropherogram for the patient is placed above the control. (C) Histology of gonad sections (400×) showing well differentiated seminiferous tubules with thick basement membrane (1) surrounded by fibroblasts (2), interstitial space filled with Leydig cells (3), spermatogonium (4) spotted in the seminiferous tubules having lumen filled with Sertoli cells (5).

sitivity syndrome [10]; however, the functional consequences of the mutation have not been analyzed. We undertook the present study to look at the etiology of the 46,XY disorder of sexual development with partial androgen insensitivity syndrome.

2. Materials and methods

2.1. Subjects and clinical history

The subject was recruited through the Institute of Reproductive Medicine (IRM), Kolkata, India, in January 2006. The patient approached the clinic with primary complaint of absence of menarche at the age of 16 years. A detailed history of the patient was taken along with physical examination. Physical examination revealed poorly developed breasts, ambiguous genitalia with a small (1 cm) phallus/penis like structure between the partially developed labial folds, separate vaginal and urethral openings, normal pubic and axillary hair (Fig. 1A). Ultrasound followed by laparoscopic surgery of the pelvic region revealed rudimentary uterus, no fallopian tubes, normal and well distended urinary bladder, abdominal gonads and no mass lesion in pelvis. Testosterone level was in upper normal range at 23.9 nmol/L (reference: 15.15–24.51); LH and FSH were elevated at 49.60 mIU/L (reference: 7–24 mIU/L) and 50.2 mIU/L (reference: 4–25 mIU/L), respectively. Androgen insensitivity index (product of absolute values of T and LH) was also higher at 1185.44 than normal range (reference: 106.05–588.24). The above characteristics of the patient were consistent with partial androgen insensitivity phenotype [5]. The patient had one normal sister and reportedly no family history of AIS; however, mother's DNA could not be analyzed due to unavailability of the sample. Peripheral blood sample of the patient was collected for cytogenetic and molecular genetic analyses. A total 200 normal healthy male individuals were recruited as controls for the study. The study was approved by the Institutional Ethics Committee of the CCMB.

2.2. Cytogenetic analyses

Cytogenetic analysis was done as detailed in our earlier study [11].

2.3. Histological studies

Abdominal gonads of the patient were removed because of cancer risk, and the tissue biopsy subjected to histologic analyses as detailed in our earlier study [11].