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Research Article

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A Novel Nonsense Mutation p.L9X in the SRY Gene Causes Complete Gonadal Dysgenesis in a 46,XY Female Patient

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Abstract

Mammalian sex is determined by a gene localized on the Y chromosome known as *SRY* (sex-determining region of the Y chromosome). *SRY* is a transcription factor that plays a key role in the initiation of the cascade of male sexual differentiation. In 46,XY humans, *SRY* mutations cause complete gonadal dysgenesis (CGD) with male to female sex reversal, which results in female genitalia without testis differentiation. The aim of this study was to look for mutations of *SRY* gene in a 46,XY CGD Tunisian female patient by direct sequencing. This method allowed us to identify a novel nonsense mutation L9X, occurring within the NH2 terminal domain of *SRY*. This novel mutation led to the appearance of a premature stop codon, resulting in a truncated protein, missing the entire HMG box functional domain and the COOH terminal domain. Because of an increased risk of developing gonadoblastoma, early molecular diagnosis allows the orientation of the clinical supervision by removing the dysgenetic gonads to prevent gonadal malignancy. Furthermore, it provides valuable information for the understanding of molecular mechanisms behind the gonadal dysgenesis.

Keywords: *SRY*; Sex reversal; Gene mutation; Sex determination

Introduction

Disorders of sex development (DSD) are congenital conditions manifested as a result of disruption of either sexual determination or differentiation [1] leading to discordance between genetic, gonadal, and anatomical sex. 46,XY DSD female phenotype are clinically classified as complete gonadal dysgenesis [2]. They present a full development of unambiguous female genitalia, a well-developed Müllerian structures, the absence of secondary sexual characteristics and streak gonads. The diagnosis may not be suspected during infancy, unless cytogenetic analysis has been performed for a prenatal diagnosis or family history of a disorder of sex development (DSD). Otherwise, individuals are diagnosed when they consult for evident delayed puberty due to the lack of estrogen and progesterone production by the streak gonads, or for gonadal tumors. In fact, the risk of developing gonadal tumors is high in individuals with dysgenetic gonads and may reach 40% [3]. For this reason, molecular diagnosis of 46,XY CGD is needed to allow the orientation of the clinical supervision to prevent gonadal malignancy by removing the dysgenetic gonads.

Mutations of the sex-determining region Y (*SRY*), *SF1*, *DHH*, *WNT4* and duplication of *DAX1* are the major molecular abnormalities associated with 46,XY CGD [4]. However, *SRY* is responsible for 15% of individuals with 46,XY CGD [5]**.** This gene encodes a transcription factor which initiates a signaling cascade that ensures male sex differentiation and testis development [6]. The SRY protein is composed of 204 amino acids and contains a central high mobility group (HMG) functional domain as well as amino terminal and carboxy-terminal domains [7].

Herein, we present the clinical, endocrinological and molecular data of a Tunisian female patient with primary amenorrhoea and discuss the pathogenicity of the identified novel nonsense mutation p.L9X.

Patient and Methods

Patient

A 15 year old Tunisian female born to non-consanguineous parents, was referred to the Department of Congenital and Hereditary Diseases of the Charles Nicole Hospital in Tunis because of primary amenorrhea without history of previous illness. Her height was 174 cm and her weight was 79.4 kg. She had no breast development (Tanner stage I), no axillary hair, a rare pubic hair and her external genitalia appeared as completely female with infantile vulva and hypoplastic labia minora. The patient had no dysmorphic features. Cytogenetic studies revealed 46,XY karyotype. Endocrinologic investigations showed hypergonadotropic hypogonadism. Her serum FSH level was 96.5 mIU/mL, LH was 32.4 mIU/mL; however, her serum testosterone level was normal for a female (0.87 nmol/l). Pelvic ultrasonography and pelvic magnetic resonance imaging were performed in order to delineate the internal genital anatomy. These analyses revealed a hypoplastic uterus and bilateral dysgenetic gonads. The father of the proband was not available for clinical examination and molecular studies.

Molecular studies

Genomic DNA isolation and *SRY* amplification: After informed consent was obtained, genomic DNA was extracted from peripheral blood sample by the standard proteinase-K extraction protocol. The extracted DNA was suspended in T10E1 buffer.

PCR amplification was performed in a volume of 50 µl containing 0.4 µg of genomic DNA, 16 pmol of each nucleotide primer, 1.25 mM of each deoxynucleotide triphosphate, 1.5 mM of $MgCl_2$, 1X PCR buffer

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(promega), and 5 U of *Taq* DNA polymerase (promega). The single exon of *SRY* gene was amplified using specific primers which were selected from the bioinformatic software primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/): SRY (F) (5'-tcattacaaaagttaacgtaacaaaga-3') and SRY (R) (5'- ggcctttattagccagagaaaa-3').

Reaction mixture was heated to 95°C for 60 s, and then cycled 35 times as follows: 13 cycles: denaturing at 95°C for 60 s, annealing at 66°C for 30 s, and extending at 72°C for 30 s; 30 cycles: denaturing at 95°C for 30 s annealing at 52°C for 30 s and extending at 72°C for 30 s. Final extension was continued for 7 min at 72°C. The amplification products were analyzed by 2% agarose gel electrophoresis with presence of ethidium bromide stain.

Sequencing and sequence analysis of *SRY* gene: The amplified products were subjected to purification using Zymo Research 25 kit according to the manufacturer's protocol then the purified PCR products were sequenced using the Big Dye® Terminator Cycle Sequencing kit (Applied Biosystems). Samples were run on an automated ABI PRISM 3130 capillary sequencer (Applied Biosystems), and data were analyzed with the ABI Seq-Scape software (Applied Biosystems).

Results

By direct sequencing of *SRY* gene of a 46,XY CGD female patient, we identified a mutation consisting of a T to A transversion (c.26T>A) which gave rise to a premature stop codon instead of leucine residue at amino acid position 9 (p.L9X) (Figure 1). Sequencing the opposite strand of the PCR products confirmed this finding. Research in the Human Gene Mutation Database, showed that this mutation has not been reported to date; therefore, this result likely reflects a novel mutation.

The premature stop codon occurs within the NH_2 terminal domain and has as consequences, a truncated protein product missing a large part of the NH_2 terminal domain (amino acid 9 to amino acid 57), the entire functional HMG box domain (amino acid 58 to amino acid 137) and the entire COOH terminal domain (amino acid 138 to amino acid 204). Thus, the mutant protein is almost certainly non-functional explaining the CGD phenotype observed in our patient.

Discussion

In the present study, we describe a novel nonsense *SRY* mutation c.26T>A identified in a 46,XY CGD patient. At protein level, this mutation leads to a substitution of a leucine by a premature stop codon at the 9th codon of the N terminal domain of SRY; thus, generating a truncated protein missing a large part of the NH2 terminal domain, the entire HMG box functional domain and the COOH terminal domain. As demonstrated by the Human Gene Mutation Database and review of the literature, this mutation was not described previously and brought the total number of *SRY* gene nucleotide substitutions (missense/nonsense) to 74 (HGMD professional 2015).

SRY is the major gene required for male sex differentiation, it allows the bipotential gonad to develop as testis by activating a cascade of genes involved in male sexual development. Mutations of this gene are known to disrupt the male differentiation pathway that may result in complete or partial male to female sex reversal [8].

The majority of *SRY* mutations occurs sporadically affecting only one individual in a family; nonetheless, cases describing familial inheritance have been reported where the paternal mosaicism for the mutation provides an explanation for this inheritance [9]. However, in our case, it cannot be ascertained whether this mutations is de novo as

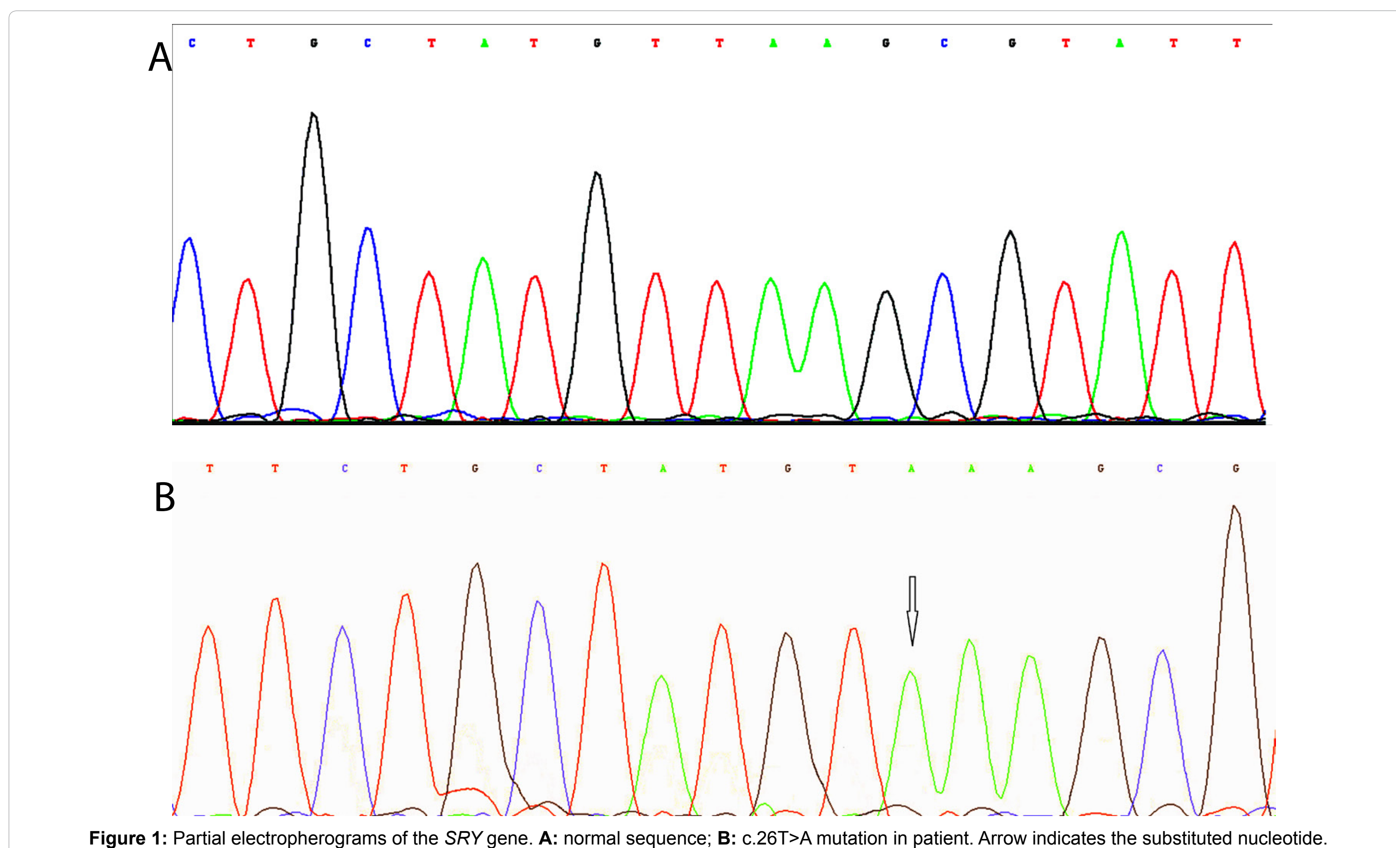

Figure 1: Partial electropherograms of the *SRY* gene. **A:** normal sequence; **B:** c.26T>A mutation in patient. Arrow indicates the substituted nucleotide.

paternal DNA was not available for analysis, and therefore, a parental germ cell mosaicism cannot be excluded.

To date, about 98 different mutations (HGMD professional 2015) responsible for approximately 15% of 46,XY gonadal dysgenesis [5] have been identified at the *SRY* open reading frame. The majority of these mutations are located within the SRY functional domain HMG box (79 amino-acids) which contains nuclear localization signal (NLS) and acts as the DNA binding domain recognizing a consensus DNA motif AACAAT [10]. This binding activity was demonstrated to induce a bend in the DNA which is necessary for testicular development [11]. In contrast, to our knowledge, only 11 mutations have been reported to lie outside the HMG box. Of these, eight are located in the 5' region upstream of the HMG box (N-terminal domain), and the remaining three lie downstream the HMG box (C-terminal domain) (HGMD professional 2015).

Analysis of Protein homology modelling realized by Gimelli et al. [12], predicted with a high possibility, that the N-terminal domain of the SRY protein could form an α-helix from amino acid in position 2 to amino acid in position 13. The remaining part of this N-terminal domain is randomly coiled [12]. The mutation L9X takes place at the middle of the α –helix and abolishes the synthesis of the following amino acids. Therefore, this mutation could almost certainly disrupt the N-terminal - α helix.

Thus, it is reasonable to suppose that if this α-helix is absent, then the SRY protein could be easily susceptible to proteolysis; could hamper the binding of SRY partners, and could play a major role in destabilizing the SRY interaction with the target DNA [12].

Besides, it has been generally accepted that the reason that the majority of the reported *SRY* mutations are clustered within the HMG box is that these are the mutations that disrupt the gene function and that other mutations are likely to be silent. However, a premature termination upstream from the HMG box would also destroy function and would not be predicted to be silent [13]. Therefore, it has been established that although the HMG box domain of the SRY is required as a DNA-protein interface, sequences outside of this domain are required to stabilize protein binding and/or generate specificity by helping to discriminate between protein partners [14].

Three XY females with CGD have been reported previously with nonsense mutations within the N-terminal domain: p.Q2X [13] p.Y4X [8] and p.Y17X [15]. The L9X mutation reported here describes the fourth novel nonsense mutation at the same domain.

The phenotype of the four XY females (including the patient of our study) is typical of patients with CGD. They present swyer's syndrome signs which include normal female external genitalia and müllerian structures, streak gonads, primary amenorrhea and absence of secondary sexual characteristics [16] except for the XY female harboring the p.Q2X mutation [13]. In fact, this woman is quite atypical since she presented to the hospital at the age of 28 years with the primary complaint of infertility and irregular menses, none of delayed puberty, as the other XY females with *SRY* mutations. The authors hypothesized that the unusual phenotype (partially functional gonads) in this case was related to the unusual mutation p.Q2X which creates a stop codon immediately following the initiating methionine and apparently inactivating her *SRY* gene [13].

In conclusion, the novel mutation described in this study highlights the key role of the *SRY* gene in the sex differentiation pathway. Because of an increased risk of developing gonadoblastoma, early molecular diagnosis allows the orientation of the clinical supervision by removing the dysgenetic gonads to prevent gonadal malignancy. Furthermore, it provides valuable information for the understanding of molecular mechanisms behind the gonadal dysgenesis.

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