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Short Communication

A novel mutation (c. 341A>G) in the *SRY* gene in a 46,XY female patient with gonadal dysgenesis

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ABSTRACT

SRY (sex-determining region Y) gene, MIM 480000, NM_005634) is crucial for sex differentiation which encodes the protein responsible for initiating testis differentiation. *SRY* mutations are associated with the presence of XY gonadal dysgenesis symptoms.
We studied a 46,XY female patient with primary amenorrhoea and negative family history. The clinical, endocrine, histopathologic and cytogenetic data are consistent with gonadal dysgenesis.
Using a molecular analysis, a novel (c.341A>G, p. N65D) missense mutation within the HMGbox of *SRY* gene was detected.
Escherichia coli expression of SRY study showed reduced expression of the mutated protein and gel retardation assay method revealed lowered DNA-binding ability in N65D variant of SRY.
The novel mutation detected in the *SRY* gene may be an aetiopathogenic factor in clinically defined 46,XY complete gonadal dysgenesis (CGD).
Because of an increased risk of gonadoblastoma, proper early diagnosis and treatment prevent development of malignancies.

Introduction

46,XY complete gonadal dysgenesis is a disorder of sex development (DSD) which results in discordance between genetic, gonadal and phenotypic sex. Several genes are involved in the process of sex differentiation, including *SRY*, *RSPO1*, *SOX9*, *NR5A1*, *WT1*, *NR0B1* and *WNT4*. The best-defined gene involved in gonadal differentiation is *SRY* located in the Y chromosome (Yp11.3) and it induces the bipotential gonad to differentiate into a testis.

The human *SRY* gene encodes a 204 amino acid protein that comprises three domains: N-terminal, central (DNA binding) and C-terminal. The central domain contains 79 amino acids and is known as a High Mobility Group (HMG) box, which is found in a number of transcription factors. This region is flanked by two nuclear localization signals (NLS) at the N- and C-terminal ends of this domain (3,4). Mutations of the *SRY* gene play a role in 46,XY disorders of sex development causes (46,XY DSD) and they are present in about 10–15% of 46,XY gonadal dysgenesis cases mutations are present (Hughes, 2008; Ostrer, 2008). The majority of detected mutations occur *de novo* within the highly conserved HMG box region, thus highlighting the critical role of this domain. *SRY* mutations can affect DNA binding, DNA bending or the nuclear transport (Harley et al., 2003).

46,XY gonadal dysgenesis individuals show a phenotypical female appearance, but they may be present at puberty with absence of secondary sexual characteristics and primary amenorrhoea. All patients with complete gonadal dysgenesis have an increased risk of malignant transformation (Cools et al., 2006; Hersmus et al., 2009).

In this study clinical, endocrinological and molecular data of a female patient with primary amenorrhoea and with a negative family history are discussed.

Case description and methods

The main complaint of the female, 18 year-old patient was primary amenorrhoea. Family history was negative. The patient's height was 167 cm. Endocrinologic investigations showed hypergonadotropic

Abbreviations: SRY, Sex-determining Region of Y chromosome; RSPO1, R-spondin 1; SOX9, SRY (sex determining region Y)-box 9; NR5A1, nuclear receptor subfamily 5, group A, member 1; WT1, Wilms tumor 1; NROB1, nuclear receptor subfamily 0, group B, member 1; WNT4, wingless-type MMTV integration site family, member 4; HMG, High Mobility Group; CGD, Complete Gonadal Dysgenesis; DSD, Disorder of Sex Development; NLS, Nuclear Localization Signal.

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hypogonadism with elevated FSH and LH and oestrogen concentration below normal reference values. Clinical examination did not confirm the presence of gonadal dysgenesis stigmata. The external genitals were female in appearance, however delayed puberty was noted (Th-1, P-1 A-0). Neither craniofacial anomalies, nor vertebral anomalies nor mental retardation were observed. A rudimentary uterus, measuring 1.72 cm × 1.23 cm, and a linear endometrium were found in intrarectal sonography. No gonadal structures were found either in the adnexal area or – bilaterally – in the inguinal fossae. A high resolution cytogenetic analysis of lymphocyte and skin fibroblast cultures showed a 46,XY karyotype. Diagnostic, laparoscopic inspection of the pelvis minor did not confirm any presence of gonads. No hysterectomy was performed, while tissue bands, which might have corresponded to rudimentary Müllerian or Wolffian derivatives, were dissected. Cytogenetic analysis of the dissected tissues showed 46,XY karyotype. A histopathological study, carried out at two independent, recognised centres, revealed the following data on the left side: rudimentary müllerian and wolffian derivatives and blood vessels, while only rudimentary wolffian derivatives and blood vessels were found on the right side. No structures, corresponding to either the testis or the ovary, even in their residual form, were found on both sides.

Molecular analysis: genomic DNA of the patient and control DNA was automatically isolated from peripheral blood lymphocytes (MagNa Pure Compact, Roche).

Y chromosomal microdeletions were analysed with multiplex-PCR using a primer set specific for these regions: Yp11.3, Yp11.2, Y cen, Yq11.22–11.23, Yq12. Molecular analysis of *SRY* gene was conducted with PCR and direct sequencing methods (Macrogen's sequencing service, Korea) (Shahid et al., 2004). Results were analysed using Mutation Surveyor Demo V3.20.

E. coli expression of SRY wild-type and mutant proteins: genomic DNA was extracted from blood samples according to the GTC method (Chomczyński, 1993). DNA encoding the SRY gene was amplified by PCR using SRY primers 5′GAGCTCGTGGATCCGAATTCCATGCAATCATAT GCTTC3′ and 5′GGCGCGCCTGTACAGAATTCCTACAGCTTTGTCCAGTG 3′. Amplification product (614 bp) was cloned into pET 44 a vector (Novagen Darmstadt Germany) using In Fusion™ 2.0 Dry Down PCR Clonning Kit. Constructs were used to transform Bl 21 (DE3) *E. coli* cells. Expression of wild-type and mutant SRY proteins were induced with 5 μM IPTG (isopropyl-1-thio-ß D-galactopyranoside) for 4 h at 37 °C. Cells were collected by centrifugation (5000 *g* for 10 min at 4 °C), suspended in lysis buffer (300 mM NaCl, 20 mM Tris-HCl, pH 8.0, 20 mM imidazole) and incubated on ice for 10 min. The suspension was sonicated and centrifuged (18,000 *g* at 4 °C). The amount of protein in supernatant was estimated with the Bradford method (Bradford, 1976). Proteins were separated with the use of 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Gel retardation assay: to investigate the effect of the c.A341>G mutation on the SRY DNA-binding activity gel retardation assay was performed. The oligonucleotide sense 5′CCGGGTTAACAGAACAATG GGTCTGGT3′ and antisense 5′ACCAGACCCATTGTTCTGT TAACCCGG 3′ were annealed to generate a double-stranded probe and end-labeled with [α-^{32}P]dATP and Sequenase 2.0 (Amersham Bioscience Munich, Germany). Labeled oligonucleotide was purified by 7% polyacrylamide gel electrophoresis in 0.5× Tris–borate/EDTA. Approximately 5 × 10^4cpm of the purified probe was used for assay. Aliquots of 10 μg of bacteria proteins (containing the same amounts of wild and mutated cell extract) were mixed with 2 μg non-specific competitor poly (dI:dC0 (Promega) in 10 ml buffer 10 mM Tris–HCl, pH 7.5, 1 mM $MgCl_2$, 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 12% glycerol and incubated at room temperature for 15 min. DNA-protein complexes were formed with 20 fmol (100,000 cpm) of the [^{32}P]-5′-end labeled oligonucleotide for 20 min at room temperature. All resulting protein-DNA complexes were loaded onto a low ionic strength, non-denaturing 7% polyacrylamide gel (30:1 cross-linking ratio) containing 6.7 mM Tris–HCl, pH 7.5; 3.3 mM sodium acetate and 1 mM EDTA. Electrophoresis was performed at 25 mA until suitable separation was achieved. Gels were vacuum-dried and autoradiographed with intensifying screens at −20 °C for 2 to 18 h.

Results and discussion

A novel *SRY* gene mutation was detected in 46,XY adult woman patient with complete gonadal dysgenesis. Y chromosomal microdeletions analysis with multiplex PCR method showed the presence of all the examined sequences. Direct sequencing of the whole *SRY* coding region revealed A to G transition at nucleotide position 341, a fact which was not previously observed (Fig. 1). This mutation, located within the conserved HMG-box region, changes asparagine to aspartic acid at codon (p.N65D).

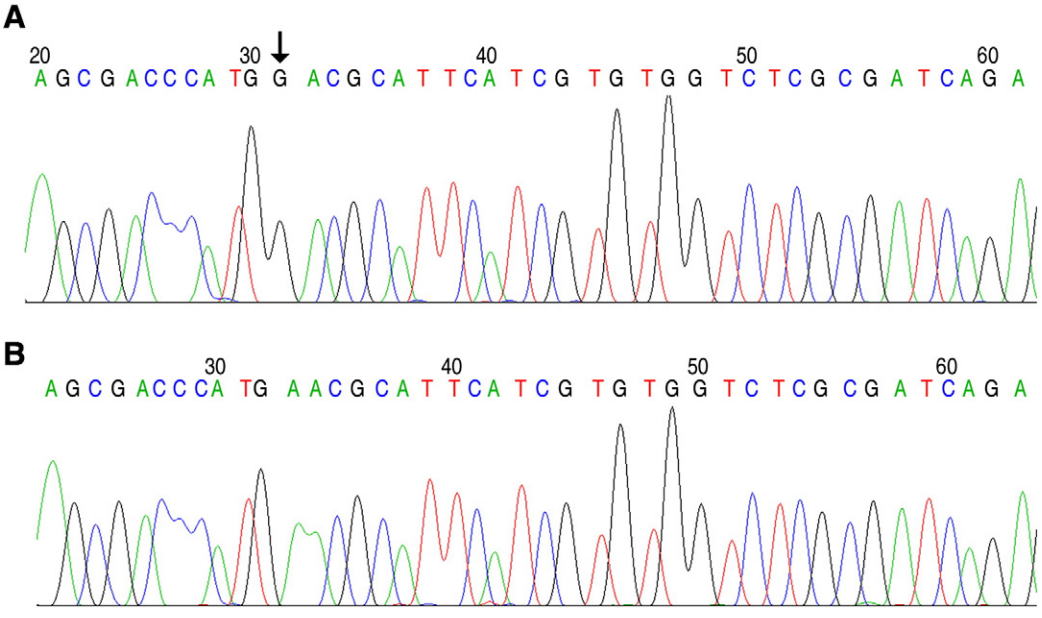

Fig. 1. Partial electropherograms of the *SRY* gene. A: c.A341>G mutation in patient; B: normal sequence. Arrow indicates the substituted nucleotides.