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BRIEF REPORT — MUTATION REPORT

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A novel missense mutation in the HMG box region of the SRY gene in a Japanese patient with an XY sex reversal

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Abstract The sex-determining region of the Y chromosome, the *SRY* gene, located on the short arm of the Y chromosome, is appreciated as one of the genes that is responsible for directing the process of sex differentiation. To date, 34 different mutations, including 29 missense and nonsense mutations in the *SRY* gene, have been described in XY female patients. We investigated the molecular basis of the sex reversal in one Japanese XY female patient by determining the nucleotide sequence of the *SRY* gene, using polymerase chain reaction and direct sequencing. We identified a novel mutation, of the substitution of Tyr for Asn at nucleotide position 87 (N87Y). This Asn residue is located within the DNA-binding high-mobility-group (HMG) motif, which is considered to be the main functional domain of the SRY protein. Further, this amino acid, Asn, is a conserved residue among mammalian *SRY* genes. These findings indicate that this amino acid substitution may be responsible for the sex reversal in this patient.

Key words 46, XY Female · Gonadal dysgenesis · SRY · HMG box · Missense mutation

Introduction

Identification of mutation(s) in the gene(s) that direct the process of sex differentiation in humans gives insights into the understanding of the molecular basis of sex differentiation. The gene for the sex-determining region of the Y chromosome (*SRY*) was the first gene identified in this process (Sinclair et al. 1990). This gene has a unique structure in which the deduced 80-amino acid region of the SRY protein shows high homology to a motif within the high-mobility-group (HMG) proteins. This conserved motif, known as the HMG box, possesses sequence-specific DNA-binding activity and acts as a transcriptional regulator in the cascade of sex determination (Gubbay et al. 1990). The XY female is rare and is characterized by sexual infantilism, the absence of differentiated gonads (only streak gonads being present), and the presence of normally developed Mullerian structures, including uterus and fallopian tubes, in a phenotypic female with an XY karyotype. Approximately 15% of XY female phenotype patients harbor nonsense or missense mutations and deletion mutations of the *SRY* gene, which are mainly present within the HMG box (Hawkins et al. 1992a; Ferguson-Smith 1992). We experienced a phenotypic female patient presenting with abdominal tumor; molecular analysis identified a novel missense mutation within the HMG box of the *SRY* gene.

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Patient and methods

Case history

A 12-year-old phenotypic female was referred to the Pediatric Department at Hokkaido University Hospital because of a right lower abdominal mass. She was 153 cm tall (+0.65 SD for normal Japanese female) and her weight was 35.9 kg. She was born to healthy unrelated parents and had had an uneventful gestation period. She had been healthy until this visit, when an abdominal mass was observed. She had not manifested any secondary sexual characteristics at that time.

On examination, a 20 × 18-cm firm mass was palpated in the lower abdomen. The patients pubertal stage was B1, PH1 according to the Tanner stage. Abdominal computed tomography detected a right gonadal mass of 18 × 11 × 7 cm, a left streak gonad, and prepubertal-size uterus. Endocrinological examination demonstrated extremely elevated basal plasma levels of follicle-stimulating hormone (24.8 mIU/ml; standard range for prepubertal teen-aged girl, 1.16–3.65 mIU/ml) and luteinizing hormone (143.0 mIU/ml; standard range for prepubertal teen-aged

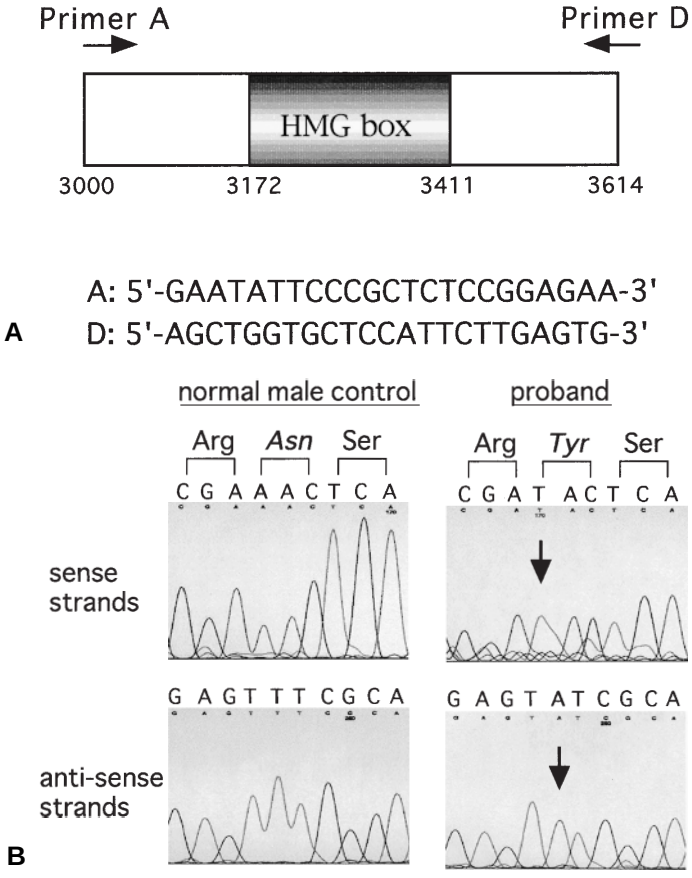

Fig. 1. **A** Location of oligonucleotides used for polymerase chain reaction and direct sequencing in the *SRY* gene. Numbers refer to GenBank Accession L08063 NID g347961. **B** Part of the nucleotide sequence of the *SRY* gene. *Arrows* indicate A-to-T nucleotide transition, resulting in amino acid substitution of Tyr for Asn. *HMG*, High-mobility-group (motif)

girl, 0.02–0.11 mIU/ml). Her karyotype was 46, XY. No mosaicism or structural anomalies of the Y chromosome were identified.

She underwent surgery for the resection of the right gonadal tumor and the left streak gonad. Histological examination revealed that the right gonadal mass was gonadoblastoma. Based on these findings, she was diagnosed as an XY female.

DNA analysis

Informed consent for DNA analysis was obtained from the patient's parents. DNA was prepared from white blood cells, using standard techniques. Primers for polymerase chain reaction (PCR) were selected in the SRY region to flank the entire motif of the HMG box, as described previously (Tajima et al. 1994) (Fig. 1A). PCR and direct sequencing of PCR products were performed using a Dye Terminator Amplitaq FS kit (Applied Biosystems, Foster City, CA) and analyzed with an ABI 373A automated sequencer (Applied Biosystems), according to a previous method (Nakae et al. 1996).

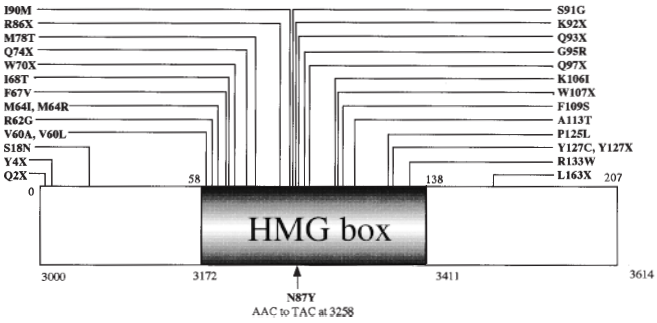

Fig. 2. The *SRY* gene and its nonsense and missense mutations. The *black bar* indicates the HMG box. The nonsense and missense mutations of the *SRY* gene reported previously are shown above the schema of the *SRY* gene. The mutation within the HMG box in our patient is shown by the *arrow* below the schema of the *SRY* gene

The protocol was approved by the institutional review board.

Results

Direct sequencing revealed the transition of adenine (A) nucleotide to thymine (T) at nucleotide position 3,258 within the HMG box (Fig. 1B). By this nucleotide transition, codon AAC [Asn (N)] at amino acid position 87 changed to TAC [Tyr (Y)]. This nucleotide transition was not detected in DNA from the patient's parents, indicating that this was a de-novo mutation. To rule out that the possibility that this mutation was not merely a polymorphism, we analyzed the *SRY* gene in 50 unrelated normal Japanese males by PCR-direct sequencing. This mutation was not present in any of these control samples.

Discussion

We identified a novel missense mutation (N87Y) within the HMG box of the *SRY* gene in our Japanese patient with XY sex reversal with gonadoblastoma. To date, 29 unique missense and nonsense mutations of the *SRY* gene have been reported in XY female patients (Fig. 2) (Hawkins et al. 1992a; Hawkins et al. 1992b; McElreavy et al. 1992a, McElreavy et al. 1992b; Muller et al. 1992; Affara et al. 1993; Jager et al. 1993; Hawkins 1993; Schmitt-Ney et al. 1993; Zeng et al. 1993; Iida et al. 1994; Poulat et al. 1994; Tajima et al. 1994; Hiort et al. 1995; Bilbao et al. 1996; Cameron and Sinclair 1997; Veitia et al. 1997; Brown et al. 1998; Domenice et al. 1998; Scherer et al. 1998). Most of these mutations are located within the HMG box domain in the SRY gene, which has a sequence-specific DNA-binding activity. This HMG box has a critical function as a transcriptional regulator in the cascade of sex determination (Werner et al. 1995). The N87Y mutation identified in our patient is also located in the HMG box. While the

Asn at codon 87 is not strictly conserved among the different HMG domain proteins, it is the most frequently found residue at this position. Thus, this Asn is presumed to be important for the normal function of SRY protein. The two mutations of I90M and S91G have also been detected near N87Y in XY female patients (Hawkins et al. 1992b; Schmitt-Ney et al. 1993). These two mutants are localized in helix 2 in the deduced tertiary structure of rat and hamster HMG1 (Read et al. 1993; Weir et al. 1993). One of these mutants, S91G, was shown to manifest reduced DNA-binding activity by an in-vitro expression study (Schmitt-Ney et al. 1993). Since N87Y is also located in helix 2, like I90M and S91G, these three mutants would, presumably, have similar biological functions, by disturbing the SRY-DNA interaction. Thus, it is most plausible that this mutation is responsible for the sex reversal in our patient, whereas the function of mutated SRY N87Y protein remains to be determined.

It is known that XY females have a risk of developing tumors of gonadal origin (Ferguson-Smith 1991). Gene(s) other than the *SRY* gene are suspected to responsible for the pathophysiology of gonadoblastoma, although the gene(s) are not yet cloned. Our patient already had a gonadoblastoma at the age of 12 years. Therefore, it is recommended that XY females should be identified prepubertally, because delayed diagnosis may bring about the development of a gonadal tumor.

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