CASE REPORT

Hypogonadotropic hypogonadism in an adult female with a heterozygous hypomorphic mutation of SOX2

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Abstract

Objective: Heterozygous SOX2 mutations have recently been reported to cause isolated hypogonadotropic hypogonadism (HH), in addition to ocular and brain abnormalities. Here, we report a further case with a heterozygous hypomorphic SOX2 mutation and isolated HH.

Patient: The patient was a 28-year-old Japanese female with congenital right anophthalmia and poor pubertal development, who was found to have HH by a gonadotropin-releasing hormone test (peak serum LH, 2.3 mIU/ml; peak serum FSH, 2.9 mIU/ml). Other pituitary hormones were normal.

Methods: We performed mutation analysis of SOX2 and functional studies of mutant SOX2 protein using the core enhancer sequence of the chicken δ-l-crystallin gene (DC5) and that of the mouse nestin gene (Nes30).

Results: A heterozygous missense mutation (224T→A, Leu75Gln) was identified in the DNA-binding domain. The mutant SOX2 protein had a severely reduced (approximately 10%) DNA-binding affinity and a markedly diminished (20–30%) transactivation potential with no dominant negative effect.

Conclusions: The results provide further support for the positive role of SOX2 in the regulation of gonadotropin production.

Introduction

Hypogonadotropic hypogonadism (HH) is a genetically heterogeneous condition defined by the deficiency of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion (1). It can occur as an isolated form or in association with other pituitary hormone deficiency. To date, several causative genes have been identified in isolated HH, including GNRHR, GPR54, DAX1, SF-1, KAL1, and FGFR1 (2, 3). Of these, mutations of GNRHR and GPR54 are free from clinical features other than isolated HH, whereas those of the remaining genes are usually associated with characteristic clinical phenotypes in addition to isolated HH (2, 3). However, mutations of such genes account for a relatively small fraction of patients with isolated HH (1), and underlying genetic factors remain to be elucidated in many patients with this condition.

SRY-related high mobility group (HMG) box gene 2 (SOX2) is a single exon gene encoding a member of SOX transcription factor family involved in the regulation of embryonic development and in the determination of cell fate (4–6). SOX proteins bind specific DNA sequences through their HMG domain, and regulate specific downstream target genes by interacting with a variety of partner proteins (6). SOX2 is expressed in multiple developing tissues including the eyes and the central nervous system (CNS) (5, 7, 8), and paired box gene 6 and brain 2 act as partner proteins of SOX2 in transcriptional regulation during the lens and the CNS development respectively (8, 9). Consistent with this, heterozygous loss-of-function mutations of SOX2 are known to cause ocular and CNS abnormalities (10, 11).

Recently, Kelberman et al. (12) have reported that heterozygous SOX2 mutations cause anterior pituitary hypoplasia and apparently isolated HH. Six patients with functionally impaired de novo SOX2 mutations invariably had HH, and exhibited clinical features consistent with HH such as micropenis and/or cryptorchidism in affected males and delayed or lack of pubertal development in affected females. Consistent with this, micropenis and/or cryptorchidism have previously been described in some male patients with SOX2 mutations (10, 11).

However, there has been no other report documenting the association between a SOX2 mutation and HH. Here, we report a further case with the association.
Subject and methods

Case report

This Japanese female patient was born at 36 weeks of gestation, with a birth length of 43.0 cm (−2.8 S.D.) and birth weight of 2.2 kg (−2.2 S.D.). She had congenital right anophthalmia, and received cosmetic repair with an artificial eye at 3 years of age. The non-consanguineous parents and the younger brother were clinically normal.

At 28 years of age she was referred to us, because of primary amenorrhea. Her height was 152.3 cm (−1.1 S.D.) and her weight 45.5 kg (−1.0 S.D.). Physical examination showed poor pubertal development (breast, Tanner stage 1; pubic hair, Tanner stage 2) with no virilization. Endocrine studies indicated isolated HH (Table 1), and her bone age was assessed as 15 years by the TW-2 method standardized for Japanese (15). Chromosome analysis revealed a 46,XX karyotype in all the 50 lymphocytes examined. Brain-computed tomography delineated right anophthalmia and apparently normal left eye and pituitary gland. The visual acuity of her left eye was 0.4 with a naked eye and 1.2 with a glass. Abdominal ultrasound studies indicated hypoplastic uterus and failed to detect ovaries. After consultation, she received oral hormone replacement therapy rather than gonadotropin therapy. She worked as a cook and had apparently normal mental development, although the measurement of intelligence quotient was refused.

Mutation analysis

This study was approved by the Institutional Review Board Committees at National Center for Child Health and Development and Osaka University. After obtaining written informed consent, leukocyte genomic DNA of this patient was amplified by PCR for the single coding exon and flanking UTRs of SOX2, using the primers shown in Table 2. Subsequently, the PCR products were subjected to direct sequencing on a CEQ 8000 autosequencer (Beckman Coulter, Fullerton, CA, USA). To confirm a heterozygous mutation, the corresponding PCR product was subcloned with TOPO TA Cloning Kit (Invitrogen), and normal and mutant alleles were sequenced separately. For controls, DNA samples of 100 normal individuals were utilized with permission.

DNA-binding assay

DNA-binding affinity of SOX2 HMG domain was examined for wildtype (WT) and mutant (MT) proteins using the core enhancer sequence at intron 3 of the chicken β-1-crystallin gene (designated as DC5) harboring a SOX2 protein binding site (9). The detailed methods have been reported previously (9). In short, WT and MT SOX2 proteins (amino acids 2–184) tagged with 6xHis at the C-terminus were expressed in Escherichia coli as glutathione S-transferase (GST)-fusion proteins using expression vector pGEX-6P-1 (Amersham Pharmacia), and the GST tag was removed with PreScission protease. Varying amounts of the recombinant SOX2 proteins were incubated with the 32P end-labeled DC5 and subjected to gel electrophoresis.

Transactivation analysis

Transactivation potential was examined with the Dual Luciferase Reporter Assay system (Promega), using DC5 containing the binding sites for SOX2 and chicken Pax6 proteins (9) and the core enhancer sequence at intron 2 of the mouse nestin gene (designated as Nes30) harboring the binding sites for SOX2 and mouse Brn2 proteins (8). The detailed methods were as described in Table 1: Summary of blood endocrine data.

<table>
<thead>
<tr>
<th>Patient data</th>
<th>Reference data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Stimulated</strong></td>
</tr>
<tr>
<td>Luteinizing hormone (LH; mIU/ml)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Follicle-stimulating hormone (FSH; mIU/ml)</td>
<td>0.4</td>
</tr>
<tr>
<td>Growth hormone (GH; ng/ml)</td>
<td>1.9</td>
</tr>
<tr>
<td>Adrenocorticotropic hormone (ACTH; pg/ml)</td>
<td>39</td>
</tr>
<tr>
<td>Thyroid-stimulating hormone (TSH; mU/ml)</td>
<td>0.6</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>12.3</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>10</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Insulin-like growth factor-I (ng/ml)</td>
<td>220</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td>18</td>
</tr>
<tr>
<td>Free thyroid (mg/dl)</td>
<td>3.12</td>
</tr>
<tr>
<td>Free triiodothyronine (ng/dl)</td>
<td>1.56</td>
</tr>
</tbody>
</table>

Stimulated values represent (1) peak values during a gonadotropin-releasing hormone test (100 µg bolus i.v.), b a growth hormone-releasing hormone test (100 µg bolus i.v.), c a corticotropin-releasing hormone test (100 µg bolus i.v.), and d a thyrotropin-releasing hormone test (500 µg bolus i.v.) (blood sampling at 0, 30, 60, 90, and 120 min); (2) the values after human menopausal gonadotropin stimulation (150 IU i.m. for 3 consecutive days) and human chorionic gonadotropin stimulation (5000 IU i.m. for 3 consecutive days) (blood sampling on day 4); and (3) the value after ACTH stimulation (250 µg i.v.) (blood sampling at 30 min). Reference data indicate the normal ranges in adult Japanese females (13, 14); those for LH and FSH indicate values at a follicular phase.
previously (8, 9). In brief, expression vectors for SOX2 (WT and MT), chicken Pax6, and mouse Brn2 were generated by inserting corresponding cDNAs into pCMV/SV2 vector (8), and luciferase reporter constructs were created by inserting DC5 and Nes30 into pδ51LucII vector with the δ1-crystallin promoter (8, 16). Subsequently, chicken embryo liver cells were transfected with (1) the reporter vector containing DC5 and the expression vectors for SOX2 (WT, MT, and WT plus MT) and Pax6, and (2) the reporter vector containing Nes30 and the expression vectors for SOX2 (WT, MT, and WT plus MT) and Brn2, together with pβG-TK vector (Promega) used as an internal control for the transfection. Luciferase assays (≥ 3 times) were performed at 48 h after the transfection.

### Results

#### Mutation analysis

A heterozygous transversion (224T>A) resulting in a substitution of the 75th leucine codon with a glutamine codon (L75Q) was identified in the helix II of DNA-binding HMG domain (Fig. 1). This missense mutation was absent in the 100 control subjects.

#### DNA-binding assay

The binding affinity of the MT SOX2 protein was severely reduced, as compared with that of the WT SOX2 protein (Fig. 2). Since 5 ng of the MT SOX2 protein gave similar intensity of the shifted band to 0.5 ng of the WT SOX2 protein, the DNA-binding affinity of the MT SOX2 protein was assessed as approximately 10% of that of the WT SOX2 protein.

#### Transactivation analysis

The transactivation potential of the MT SOX2 protein was markedly decreased, as compared with that of the WT SOX2 protein (approximately 20% for DC5 and approximately 30% for Nes30; Fig. 3A and B). Consistent with the severely attenuated (approximately 10%) DNA-binding affinity, the activation levels were similar between the assays using 2 ng of the MT SOX2 expression vector and those using 0.2 ng of the WT SOX2 expression vector, for both reporters with DC5 and Nes30. Furthermore, the activation levels were comparable between the assays with WT SOX2 protein only and those with WT plus MT SOX2 proteins for both reporters, indicating the lack of a dominant negative effect of the MT SOX2 protein.

### Table 2

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward primer; reverse primer</th>
<th>Location (bp)*</th>
<th>AT (C); PS (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOX2-1</td>
<td>CCGCATGTACAACATGATGGA; TTAGCCTCGTGATGACG</td>
<td>−4 ~ +17; +266 ~ +284</td>
<td>60; 288</td>
</tr>
<tr>
<td>SOX2-2</td>
<td>GAAACCTTTTGGACGACCGGA; ATCATGCTGTAGCTGCCTT</td>
<td>+237 ~ +257; +502 ~ +521</td>
<td>60; 285</td>
</tr>
<tr>
<td>SOX2-3</td>
<td>ACAGTTACGCGCCGATGAA; ATGCTGATCATGTCCCGGA</td>
<td>+473 ~ +491; +809 ~ +827</td>
<td>60; 355</td>
</tr>
<tr>
<td>SOX2-4</td>
<td>ATGCACCCTACGACGTGA; CCAAAAAGAAGTCCAGGATC</td>
<td>+589 ~ +607; +1190 ~ +1209</td>
<td>60; 621</td>
</tr>
</tbody>
</table>

AT, annealing temperature; PS, product size.

*The coding sequence for SOX2: +1 ~ +951.

Figure 1 Mutation analysis of SOX2 showing a heterozygous missense mutation (224T>A, L75Q). The mutation has been indicated by the direct sequencing, and confirmed by the subsequently performed sequencing of the subcloned normal and mutant alleles. The L75Q mutation resides at the HMG (high mobility group) domain with a DNA-binding capacity.

Figure 2 Electrophoretic mobility shift assay using 32P end-labeled DC5 and the wildtype (WT) and the L75Q mutant SOX2 proteins.
Discussion

A heterozygous L75Q mutation was identified in the HMG domain of SOX2 in this patient. Furthermore, the MT SOX2 protein was shown to have reduced DNA-binding affinity and decreased transcription activity with no dominant negative effect. These findings suggest that the L75Q mutation is a hypomorphic mutation retaining a residual activity.

This patient had unilateral anophthalmia and apparently normal mental development. This mild phenotype would primarily be due to the residual SOX2 activity. In addition, other genetic and environmental factors would also be relevant to the phenotypic consequences, because the ocular and CNS phenotype is not necessarily dependent on the residual activity alone (12). Furthermore, the degree of residual SOX2 activity and the status of other factors would also be involved in the development of several infrequent features such as esophageal atresia, sensorineural deafness, and short stature (10–12, 17, 18).

The salient feature of this patient is isolated HH. This provides further support for SOX2 being involved in the regulation of gonadotropin production. In this context, several findings are noteworthy. First, she had obvious HH in the presence of relatively mild ocular lesion and apparently normal CNS function. This would primarily be compatible with the phenotype of heterozygous Sox2 knockout mice exhibiting pituitary dysfunction and normal ocular development (12), and may suggest that gonadotropin production is more sensitive to the reduced SOX2 function than ocular and CNS development. Second, there was no anterior pituitary hypoplasia. This implies that SOX2 mutations do not necessarily lead to pituitary hypoplasia. Third, other pituitary hormones were normal, as in the patients described by Kelberman et al. (12). This suggests that the gonadotropin is most vulnerable to reduced SOX2 dosage among pituitary hormones. However, it should be pointed out that heterozygous Sox2 knockout mice have multiple pituitary hormone deficiency (12), and that SOX2 protein is capable of transactivating HESX1 (12, 19), a causative gene for panhypopituitarism and optic nerve abnormality (20). Thus, in conjunction with anterior pituitary hypoplasia in most patients with SOX2 mutations (12), heterozygous SOX2 mutations could affect other pituitary hormones in exceptional patients.

It remains to be clarified how SOX2 mutations lead to HH. However, it has been reported that murine Sox2 expression is identified in the presumptive hypothalamus and the Rathke’s pouch at E11.5, whereas it is confined around the Rathke’s pouch lumen at a later age and detected in some non-endocrine cells only in the adult pituitary (12). Thus, HH in SOX2 mutations may primarily be ascribed to dysregulated hypothalamo-pituitary axis during the early life, rather than a cell-type specific dysfunction of the gonadotropes.

In summary, the results provide further support for the positive role of SOX2 in the regulation of gonadotropin production. Further studies will permit to define the pituitary phenotype and the molecular mechanism leading to HH in patients with SOX mutations.
Acknowledgements

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References


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