SOX2 nonsense mutation in a patient clinically diagnosed with non-syndromic hypogonadotropic hypogonadism

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Abstract. Hypogonadotropic hypogonadism (HH) is a genetically heterogeneous condition that occurs either as an isolated disorder or as a component of congenital malformation syndromes. SOX2 is a causative gene of syndromic HH characterized by anophthalmia, microphthalmia, or coloboma and other neurological defects such as epilepsy. To date, the causal relationship between SOX2 abnormalities and non-syndromic HH remains speculative. Here, we identified a nonsense mutation of SOX2 in a male patient clinically diagnosed with non-syndromic HH. The patient had epilepsy but no additional clinical features. Ophthalmological examination revealed no abnormalities except for decreased thickness of the retinal nerve fiber layer. Audiometry showed mild sensorineural hearing impairment of both ears. Hormonal evaluation suggested isolated gonadotropin deficiency. Next-generation sequencing-based mutation screening of 13 major causative genes for HH identified a p.Lys35* mutation in SOX2 and excluded pathogenic mutations in other tested genes. The p.Lys35* mutation appeared to encode a non-functioning SOX2 protein that lacks 283 of 317 amino acids. The SOX2 mutation was absent in the maternal DNA sample, while a paternal sample was unavailable for sequence analysis. These results expand the clinical consequences of SOX2 haploinsufficiency to include non-syndromic HH. Systematic mutation screening using a next-generation sequencer and detailed evaluation of nonspecific ocular/neurological features may help identify SOX2 mutation-positive individuals among HH patients.

Key words: Eye, Gonadotropin deficiency, Mutation, Next generation sequencer, Phenotypic variation

HYPOGONADOTROPIC HYPOGONADISM (HH) is a genetically heterogeneous condition that occurs either as an isolated disorder or as a component of congenital malformation syndromes [1-4]. Although more than 30 genes have been implicated in non-syndromic HH, mutations in these genes have been identified only in less than half of the reported cases [4, 5].

SOX2 represents one of the causative genes of syndromic HH [1, 3-12]. SOX2 encodes a transcription factor involved in the development of eyes, pituitary, and central nervous system [1, 3, 6, 13]. To date, heterozygous SOX2 mutations/deletions have been identified in more than 100 individuals [6-12, 14-21]. These abnormalities typically lead to anophthalmia, microphthalmia, or coloboma, in addition to other neurological defects such as HH, brain anomaly, intellectual disability, epilepsy, and hearing loss [6-11, 15-21]. Ocular anomalies were documented in almost all known cases with SOX2 haploinsufficiency, while HH was observed in a substantial fraction of the cases [6-12, 14-21].

In 2014, Takagi et al. have identified an SOX2 mutation (p.Tyr110Cys) in a male patient with HH [14]. The patient exhibited nystagmus, retinal detachment, and seizure, but no additional clinical features. This study provided the first indication that SOX2 mutations may underlie non-syndromic HH. However, this notion is
based on the data of a single patient, and therefore, the causal relationship between SOX2 abnormalities and non-syndromic HH remains speculative. Actually, non-syndromic HH may be an exceptional phenotype of SOX2 abnormalities specifically caused by hypomorphic missense mutations, because p.Tyr110Cys was shown to retain considerable in vitro transactivation activity [14]. Here, we report a patient clinically diagnosed with non-syndromic HH, in whom we identified a nonsense mutation of SOX2.

**Patient and Methods**

**Case report**

The patient was a 19 year-old male born to non-consanguineous Japanese parents. His parents and two siblings were phenotypically normal. The patient had an episode of seizure at 3 years of age and was treated with anticonvulsants. The drug was temporarily discontinued when the patient was 9 years of age, but was restarted seven years later because of the recurrence of seizure. His growth and development were otherwise uneventful. The patient had normal educational achievement in schools and was admitted to a college.

At 19 years of age, the patient visited our clinic because of a lack of pubertal development. He had normal stature (168 cm, -0.48 SD) and body weight (54 kg, -0.97 SD), but showed no pubertal signs; he had small testes (1 mL), short penile length (5.0 cm) and no pubic hair. His bone age was delayed (13.3 years). Magnetic resonance imaging detected no abnormality in the hypothalamus, pituitary, olfactory bulbs, ocular bulbs, or optic nerves. He had normal visual activity with mild myopia (a best-corrected vision of over 20/20). Optical coherence tomography showed decreased thickness of the retinal nerve fiber layer (Fig. 1A). Coloboma was absent. He had normal orbit size. Intravenous olfaction test indicated normal sense of smell. Audiometry suggested mild sensorineural hearing impairment of both ears (Fig. 1B). Blood gonadotropin levels were low at baseline and poorly responded to GnRH stimulation (Table 1). Blood testosterone levels were decreased, while levels of insulin-like growth factor-1 and thyroid and adrenal hormones were within the normal ranges.

**Molecular analysis**

This study was approved by the Institutional Review Board Committee at the National Center for Child Health and Development and performed after obtaining written informed consent. Genomic DNA samples were extracted from peripheral leukocytes of the patient and his mother. The patient’s sample was subjected to systematic mutation screening. The coding region of SOX2, as well as those of 12 other major causative genes of HH (CHD7, FGF8, FGFR1, GNRH1, GNRHR, KAL1, KISS1R, OTX2, PROK2, PROKR2, SOX3, and TACR3), were amplified by multiplex-PCR and sequenced on a next generation sequencer (NGS) (Kazusa DNA Research Institute, Kisarazu, Japan). Possible pathogenic mutations were confirmed by Sanger sequencing. We retrieved the obtained mutation from the previous reports or the registrations for exome databases (The ExAC browser, http://exac.broadinstitute.org/; 1000 Genome Browser, https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/; and Human Genetic Variation Browser, http://www.hgvd.genome.med.kyoto-u.ac.jp/). To clarify whether a SOX2 mutation detected in the patient was shared by the mother, we performed Sanger sequencing of the maternal DNA sample. Furthermore, to examine the possible somatic mosaicism of the SOX2 mutation identified in the patient’s leukocyte DNA, we further analyzed a DNA sample extracted from hair follicles of the patient.

**Results**

A nonsense mutation in SOX2 (c.103A>T, p.Lys35*) was detected by NGS and confirmed by Sanger sequencing (Fig. 1C). The lysine residue at the 35th codon is located in the N-terminal domain adjacent to the DNA binding HMG domain (Fig. 1C). This mutation has not been reported previously and was absent in the exome databases. The patient had no mutations in other tested genes. The SOX2 mutation was absent in the DNA sample of the patient’s mother. A sample of the father was unavailable.

The results of NGS showed that the mutant allele accounted only for 36.7% (2,022/5,510) of the total reads. Moreover, the electropherogram of Sanger sequencing showed that the signal peak of the mutant allele was slightly lower than that of wildtype allele (Fig. 1C). These data suggested possible somatic mosaicism of the SOX2 mutation identified in the patient’s leukocyte DNA, we further analyzed a DNA sample extracted from hair follicles of the patient.
SOX2 mutation and hypogonadotropic hypogonadism

Fig. 1  Clinical and molecular findings of the patient

A: Ophthalmological examinations. The patient had no anophthalmia, microphthalmia, or coloboma, but showed decreased thickness of the retinal nerve fiber layer.  
B: Pure-tone audiometry. The patient showed mild bilateral sensorineural hearing loss (pure-tone average of thresholds at 0.5, 1, 2, and 4 kHz: right, 28.8 dB; left, 28.8 dB).  
C: Molecular analysis. The patient carried c.103A>T, p.Lys35* in SOX2 (red arrows). In the leukocyte-derived DNA, the signal peak of the mutant allele (T) was slightly lower than that of the wildtype allele (A), while in the hair follicle-derived DNA, both peaks were almost equivalent. This mutation was absent in the patient’s mother.  

Table 1  Blood hormone values of the patient

|                  | LH (IU/L) | FSH (IU/L) | Testosterone (ng/mL) |
|------------------|-----------|------------|----------------------|
|                  | Basal     | Stimulated | Basal                | Stimulated | Basal   | Stimulated |
|                  |           |            |                      |            |         |            |
| Basal            | < 0.10    | 1.14       | < 0.03               | 2.34       |
| (0.79-5.72)      | (3.95-57.20) | (2.00-8.30) | (4.22-5.22) | (11.03-13.11) |
| Stimulation      |           |            |                      |            |         |            |
| Testosterone     | 0.36      | 2.50       |                      |            |         |            |
| (2.00-8.30)      | (4.00-24.90)|            |                      |            |         |            |
| Reference values of adult males are shown in parentheses. Hormone values below the reference range are boldfaced.  

*a* GnRH stimulation test (100 µg bolus i.v.; blood sampling at 0, 30, 60, 90, and 120 minutes).  
*b* Human chronic gonadotropin stimulation test (5,000 IU i.m. for 3 days; blood sampling on day 6).
Discussion

We identified a hitherto unreported SOX2 mutation in a male patient with HH who had no additional clinical features except for a thin retinal nerve fiber layer, mild hearing impairment, and epilepsy. The p.Lys35* mutation of SOX2 likely encodes a non-functioning protein, because it deletes 283 of 317 amino acids. To date, only one missense mutation with substantial residual activity (p.Tyr110Cys) has been linked to non-syndromic HH [14]. Our data indicate for the first time, that complete loss-of-function of one SOX2 allele can underlie HH without apparent ocular malformations. Decreased thickness of retinal nerve fiber layer, mild hearing impairment, and epilepsy in our patient may reflect suboptimal SOX2 function in the eye and central nervous system, respectively. This case may represent an extremely mild manifestation of SOX2 haploinsufficiency. Our findings are consistent with those of animal studies, which documented normal ocular development and reduced expression of luteinizing hormone protein in the pituitary of heterozygous Sox2 knockout mice [6]. Furthermore, Dennert et al. identified SOX2 abnormalities in three patients who had neuronal defects but no major eye malformations [12]. These results argue against the concept that during development, eyes are more vulnerable to reduced SOX2 levels than other tissues [6, 22]. It appears that the phenotypic variations of SOX2 abnormalities vary more widely than previously recognized.

There are two possible explanations for the mild ocular phenotype of our patient. First, this patient may have somatic mosaicism. Indeed, sequence analysis of the lymphocyte-derived DNA sample suggested that the proportion of the mutant allele is less than 50% of the SOX2 allele. However, sequence analysis using hair follicle-derived DNA showed nearly equal amounts of the mutant and wildtype alleles. Considering that hair follicles and eyes are both of ectodermal origin whereas leukocytes are derived from the mesoderm, the results of hair follicle-derived DNA, rather than those of leukocyte-derived DNA, are closely associated with the condition of ocular tissues. Thus, although we cannot exclude the possibility that p.Lys35* in this patient is a somatic mutation, his mild ocular phenotype is unlikely to be ascribed to low-frequent somatic mosaicism. Instead, this case possibly reflects the broad phenotypic spectrum of SOX2 haploinsufficiency, as reported in previous cases [15]. Indeed, mice compound heterozygous for hypomorphic/null Sox2 alleles were shown to exhibit eye anomalies of various severities [22]. Since SOX2 participates in a broad transcriptional regulation network [23], the consequence of SOX2 haploinsufficiency appears to be modified by other transcriptional factors. Indeed, in vitro assays using murine ES cells revealed that Sox2 suppression does not necessarily result in parallel downregulation of genes with a SOX2 binding motif-containing enhancer [24]. Multiple SOX proteins, such as SOX4, SOX11, and SOX15, were shown to have an in vitro binding activity to the SOX2 binging sites [24], and therefore may play complementary roles for SOX2. However, the mechanism underlying the mild phenotype of our patient remains to be determined in future studies.

Notably, our patient was diagnosed with SOX2 haploinsufficiency only through NGS-based mutation screening. The results of this study emphasize the usefulness of NGS in the molecular diagnosis of genetically heterogeneous disorders. SOX2 abnormalities likely account for only a small percentage of HH cases, because our previous analysis has failed to identify any SOX2 mutations in 58 patients with HH [5]. Since both our patient and the patient with p.Tyr110Cys [14] manifested minor ocular changes and episodes of seizure, detailed clinical evaluation for these features may be helpful for identifying SOX2 mutation-positive individuals among non-syndromic HH patients. To date, mutation analyses of SOX2 have been performed primarily for patients with eye anomalies [6-9, 15-21]. Further studies are necessary to clarify the precise frequency and the phenotypic spectrum of SOX2 abnormalities.

In conclusion, this study expands the clinical consequences of SOX2 haploinsufficiency to include non-syndromic HH. Systematic mutation screening using NGS and detailed evaluation for minor ocular changes or epilepsy may help identify SOX2 mutation-positive individuals among HH patients.

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Disclosure

None of the authors have any potential conflict of interest associated with this research.

References

1. Kelberman D, Rizzoti K, Lovell-Badge R, Robinson IC, Dattani MT (2009) Genetic regulation of pituitary gland development in human and mouse. Endocr Rev 30: 790-829.

2. de Roux N, Carel JC, Léger J (2016) Congenital Hypogonadotropic Hypogonadism: A Trait Shared by Several Complex Neurodevelopmental Disorders. Endocr Dev 29: 72-86.

3. Tzikaﬁeri V, Kelberman D, Dattani MT (2008) The role of SOX2 in hypogonadotropic hypogonadism. Sex Dev 2: 194-195.

4. Topaloglu AK, Kotan LD (2016) Genetics of Hypogonadotropic Hypogonadism. Endocr Dev 29: 36-49.

5. Izumi Y, Suzuki E, Kanzaki S, Yatsuga S, Kinjo S, et al. (2014) Genome-wide copy number analysis and systematic mutation screening in 58 patients with hypogonadotropic hypogonadism. Fertil Steril 102: 1130-1136.

6. Kelberman D, Rizzoti K, Avilion A, Bittner-Glindzicz M, Cianfarani S, et al. (2006) Mutations within Sox2/ Sox11 microphthalmia. Am J Med Genet A 135: 1-7.

7. Chassaing N, Causse A, Viguouroux A, Delahaye A, Alessandri JL, et al. (2014) Molecular findings and clinical data in a cohort of 150 patients with anophthalmia/microphthalmia. Clin Genet 86: 326-334.

8. Suzuki J, Azuma N, Dateki S, Soneda S, Muroya K, et al. (2014) Mutation spectrum and phenotypic variation in nine patients with SOX2 abnormalities. J Hum Genet 59: 353-356.

9. Mauri L, Franzoni A, Scarcello M, Sala S, Garavelli L, et al. (2015) SOX2, OTX2 and PAX6 analysis in subjects with anophthalmia and microphthalmia. Eur J Med Genet 58: 66-70.

10. Sato N, Kamachi Y, Kondoh H, Shima Y, Morohashi K, et al. (2007) Hypogonadotropic hypogonadism in an adult female with a heterozygous hypomorphic mutation of SOX2. Eur J Endocrinol 156: 167-171.

11. Kelberman D, de Castro SC, Huang S, Crolla JA, Palmer R, et al. (2008) SOX2 plays a critical role in the pituitary, forebrain, and eye during human embryonic development. J Clin Endocrinol Metab 93: 1865-1873.

12. Dennert N, Engels H, Cremer K, Becker J, Wohlleber E, et al. (2017) De novo microdeletions and point mutations affecting SOX2 in three individuals with intellectual disability but without major eye malformations. Am J Med Genet A 173: 435-443.

13. Jayakody SA, Andoniadou CL, Gaston-Massuet C, Signore M, Cariboni A, et al. (2012) SOX2 regulates the hypothalamic-pituitary axis at multiple levels. J Clin Invest 122: 3635-3646.

14. Takagi M, Narumi S, Asakura Y, Muroya K, Hasegawa Y, et al. (2014) A novel mutation in SOX2 causes hypogonadotropic hypogonadism with mild ocular malformation. Horm Res Paediatr 81: 133-138.

15. Williamson KA, FitzPatrick DR (2014) The genetic architecture of microphthalmia, anophthalmia and coloboma. Eur J Med Genet 57: 369-380.

16. Fantes J, Ragge NK, Lynch SA, McGill NI, Collin JR, et al. (2003) Mutations in SOX2 cause anophthalmia. Nat Genet 33: 461-463.

17. Ragge NK, Lorenz B, Schneider A, Bushby K, de Sanctis L, et al. (2005) SOX2 anophthalmia syndrome. Am J Med Genet A 135: 1-7.

18. Bakrana P, Robinson DO, Bunyan DJ, Salt A, Martin A, et al. (2007) SOX2 anophthalmia syndrome: 12 new cases demonstrating broader phenotype and high frequency of large gene deletions. Br J Ophthalmol 91: 1471-1476.

19. Schneider A, Bardakjian T, Reis LM, Tyler RC, Semina EV (2009) Novel SOX2 mutations and genotype-phenotype correlation in anophthalmia and microphthalmia. Am J Med Genet A 149: 2706-2715.

20. Gerth-Kahlert C, Williamson K, Ansari M, Rainger JK, Hingst V, et al. (2013) Clinical and mutation analysis of 51 probands with anophthalmia and/or severe microphthalmia from a single center. Mol Genet Genomic Med 1: 15-31.

21. Schilter KF, Reis LM, Schneider A, Bardakjian TM, Abdul-Rahman O, et al. (2013) Whole-genome copy number variation analysis in anophthalmia and microphthalmia. Clin Genet 84: 473-481.

22. Taranova OV, Magness ST, Fagan BM, Wu Y, Surzenko N, et al. (2006) SOX2 is a dose-dependent regulator of retinal neural progenitor competence. Genes Dev 20: 1187-1202.

23. Zhang S, Cui W (2014) Sox2, a key factor in the regulation of pluripotency and neural differentiation. World J Stem Cells 6: 305-311.

24. Masui S, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, et al. (2007) Puripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nat Cell Biol 9: 625-635.