SOX2 heterozygous mutations cause multiple extraocular phenotypes in boys

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To the Editor: The SRY-related high-mobility-group-box protein-2 (SOX2) is most notably expressed in the eye, placodes, forebrain, and hypothalamus-pituitary and is involved in early embryonic development.[1] Loss-of-function mutations or deletions in *SOX2* could lead to uni- or bi-lateral anophthalmia/microphthalmia (A/M) as well as other related disorders, such as anophthalmia/esophageal-genital syndrome. An increasing number of studies have found that *SOX2* mutations can cause variable extraocular symptoms, including growth retardation, sensorineural hearing loss, mental retardation, no pubertal signs, and male genitourinary tract malformations (micropenis, cryptorchidism, and hypospadias). Indeed, cases with *SOX2* pathogenic mutations but no or minor ocular symptoms have been reported less frequently. Several studies found that *SOX2* heterozygous mutations cause typical signs of complete hypogonadism without major ocular malformations in men or women, such as isolated hypogonadotropic hypogonadism (HH), but no other HH pathogenic gene was identified.[2,3] Therefore, our study is the first to report the cases of three Chinese patients with *SOX2* mutations referred due to micropenis and/or cryptorchidism combined with craniofacial deformities or intellectual disability.

We report three patients who visited the endocrinology clinic because of micropenis and/or cryptorchidism. The characteristics of the cases were summarized and compared with those reported in the literature. The hormone detection, human chorionic gonadotropin (hCG) standard and prolonged stimulating test and luteinizing hormone releasing hormone (LHRH) stimulating test were performed according to our previous study.[4] NGS was completed by the Kangxu Company (Beijing, China). Patient 1 underwent panel sequencing, including 167 genes involved in gonadal development, and both patients 2 and 3 underwent whole-exome sequencing. Sanger sequencing was used to verify the mutations in samples from the same family. Mutations with a minor allele frequency of <1% in East Asian people are presented. We checked the pathogenicity of the mutations according to the American Society of Medical Genetics and Genomics.

When patient 1 was 6 months at the first visit, he had micropenis (the penis was 2.3 cm long and 1.2 cm in diameter) without any deformity or mental or physical development disorders. Hormone levels at 3.4 years old were as follows: anti-Mullerian hormone (AMH) > 23.00 ng/ml, inhibin B (INHB) 126.20 pg/ml, and testosterone (T) 434 ng/dl after the hCG stimulation test. The LHRH stimulation test was performed in our hospital when the patient was 3.5 years old, and the results were as follows: basic luteinizing hormone (LH) 1.89 IU/L, follicle stimulating hormone (FSH) 5.48 IU/L, T <20 ng/dl, and peak LH/FSH = 3.47/5.48 = 0.63>0.60, suggesting a normal pituitary response. He was detected to harbor *SOX2* mutation (p.T232N) through a molecular genetics test. Given the age of the patient, HH was suspected. He took testosterone undecanoate to treat micropenis for 1 month, and the penis grew to 4 cm long and 1.5 cm in diameter. The patient was followed up for 3.5 years. His hearing, smell, and vision were normal. Patient 2 was 2.6 years old at the first visit. He carried the same *SOX2* gene mutation as patient 1, and manifested bilateral cryptorchidism and craniofacial deformities (normal eyes, fanning ears, no inner cochlea, low nasal bridge, high arch bow, crooked mouth when crying, and curved fifth finger). The basic sex hormone levels of patient 2 at the first visit when he was 2.6 years old were as follows: LH 0.12 IU/L, FSH 0.34 IU/L, T < 20 ng/dL, AMH 16.63 ng/mL, INHB 27.63 pg/mL, and T 25 ng/dL after the hCG prolonged test, which suggested

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testicular dysplasia. Currently, the patient is 3.2 years old, with normal intelligence, hearing, smell, and vision and no abnormalities in ocular fundus examination. The mothers of both patients 1 and 2 are carriers of *SOX2* mutations, and both show only delayed menarche. Patient 3 was 5 months at the first visit, and he showed micropenis (the penis was 1.0 cm long and 0.6 cm in diameter), bilateral cryptorchidism, mental retardation, and eye and craniofacial deformities (sluggish face, flat nose, left ptosis, poor muscle strength at the back and neck, high palatal arch, decreased muscle strength of the limbs, and fat pad of two fingers). The basic hormone levels of patient 3 at 5 months were as follows: LH 1.40 IU/L, FSH 7.90 IU/L, T < 20 ng/dL, T 116 ng/dL after hCG stimulation test, AMH > 23.00 ng/mL, INHB 74.50 pg/mL, insulin-like growth factor-1 (IGF-1) 50.9 ng/mL, and peak LH/FSH = 2.59/7.90 = 0.33<0.60 after the LHRH stimulation test, which suggested the pituitary gland could have a response. Thyroid function, adrenocorticotropic hormone and cortisol were normal; hearing, smell, and vision were normal; and no abnormalities were found in ocular fundus examination. The hormone levels and LHRH stimulation test results are shown in Supplementary Tables 1, http://links.lww.com/CM9/A789 and 2, http://links.lww.com/CM9/A790. Pituitary MRI of all three patients showed that the pituitary gland was normal, and the olfactory bulb, olfactory tract, and olfactory groove developed normally. Patients 1 and 2 carry the same reported heterozygous pathogenic site mutation (p.T232N) in the *SOX2* gene, which is located in the carboxy terminal transcription activation region, and patient 3 carries a *de novo* nonsense heterozygous mutation (p.Y110X) that has not been reported in the literature, but a missense mutation (p.Y110C) at the same site has been reported to cause non-syndromic HH in a male patient.[5] Interestingly, we found that all three patients carried another HH-related gene variant: *FGFR1* c.238C>T/p.R80C from the father in patient 1; a *de novo CHD7* c.2656C>T/p.R886W mutation in patient 2; and *SEMA3A* c.1432G> A/p.E478K from the mother in patient 3 [Supplementary Figure 1, http://links.lww.com/CM9/A788].

In 2003, the *SOX2* gene mutation was first reported in patients with A/M deformities. To date, a total of 123 cases have been reported, including males (57/109, 52.3%) and females (52/109, 47.7%), age at presentation ranges from 20 weeks of gestation to 65 years old, and 43.9% (25/57) of male patients showed genital abnormalities [Supplementary Tables 3, http://links.lww.com/CM9/A791 and 4, http://links.lww.com/CM9/A792]. Almost all patients chose to visit the ophthalmology clinic, except for those patients without major eye deformities. All three patients in our study were male, and they showed no major ocular symptoms.

Among the patients with *SOX2* gene mutation, 91.1% (112/123) had major ocular deformities. Among the extraocular symptoms, the most common was developmental delay/mental retardation, accounting for 40.7% (50/123), followed by brain anomaly, accounting for 28.5% (35/123); motor development delay, 22.0% (27/123); male genital abnormalities (including micropenis, cryptorchidism, and hypospadias), 20.3%(25/123); short stature (SS), 17.1% (21/123); facial dysmorphism, 12.2% (15/123); and non-syndromic HH, 4.9% (6/123). The clinical and genetic features of all patients are listed in Supplementary Table 5, http://links.lww.com/CM9/A793. The three patients in the study were mainly genital abnormalities, left ptosis, hypotonia, SS, and intellectual impairment, but with no major ocular abnormalities and seizures.

By analyzing all reported mutations in *SOX2*, mutational types included frameshift (39.8%, 49/123), deletion (22.0%, 27/123), nonsense (19.5%, 24/123), and missense (18.7%, 23/123) mutations. Of these mutations, 72.6% (53/73) were *de novo*, and 15.1% (11/73) were inherited from a mother (11.0%, 8/73) or father (4.1%, 3/73) who had completely normal phenotypes; 4.1% (3/73) of variants were inherited from a mother who had germinal mosaicism but normal phenotypes; and 5.5% (4/73) were inherited from a parent who manifested abnormal syndromes, including one father with ocular deformity and one mother with HH.[6] In our three patients, mutations in patients 1 and 2 were inherited from the mother, who showed only delayed puberty, and the mutation in patient 3 was *de novo*.

In Supplementary Table 5, http://links.lww.com/CM9/A793, we present 11 cases with *SOX2* mutations but without major ocular deformities. Of our more than 500 cases of genetically positive 46, XY disorders of sex development (DSD) in our single center, only 3 *SOX2* mutations (6‰) were identified, suggesting that the incidence of *SOX2* gene mutations is lower in DSD patients without major ocular deformities. In our study, we report 3 patients with micropenis, cryptorchidism, and/or hypospadias as the main phenotypes but no significant ocular deformity. It is noteworthy that both patients 1 and 2 carry the same mutation in the *SOX2* gene (c.695C>A/p.T232N) transmitted from the mother, but the phenotypes were very different. Patient 1 showed merely micropenis, while patient 2 manifested bilateral cryptorchidism, facial deformities, and asymmetric cry faces, but both had normal vision and no ocular deformities, and their mothers had only delayed menarche. A previous study reported that in one Chinese father and son carrying the same *SOX2* mutation as our patients, the father showed ocular defects but no reproductive system abnormalities. The son manifested ocular defects, arachnoid cysts, and penoscrotal hypospadias, but there was no follow-up description of pubertal development and fertility.

In another family with a frameshift mutation in the *SOX2* gene (p. G280Afs91X), the mother was diagnosed with isolated HH due to primary amenorrhea and no secondary sexual development at the age of 18, without ocular diseases or other deformities. With the help of assisted reproductive fertility, she gave birth to one son and one daughter; the son shows anophthalmia, and the daughter has unilateral microphthalmia deformity. All of these results suggest a broad phenotypic spectrum among patients with *SOX2* mutations, and there is no obvious correlation between genotypes and phenotypes.[7] Heterozygous *SOX2* mutations in human patients commonly cause pituitary hypoplasia on imaging, usually leading to

low concentrations of LH and FSH (ie, typical HH) or growth factor (GH) deficiency and SS in some conditions. However, HH was also observed in patients without pituitary hypoplasia, and further study showed that *SOX2* mutations could reduce gonadotropin-releasing hormone (GnRH) levels and misdirect axonal migration, as evidenced by the phenomenon that HH patients carrying *SOX2* mutations could respond to GnRH stimulation. Our previous study and others showed that multiple gene defects might synergize to cause a more severe HH phenotype in at least 20% of cases.[4,8] The three patients in our study carried another HH-related gene variant: *FGFR1* c.238C>T/p.R80C from the father in patient 1, a *de novo* mutation in *CHD7* c.2656C>T/p.R886W in patient 2, and *SEMA3A*: c.1432G>A/p.E478K from the mother in patient 3. Given the symptoms, signs, and genetic results, these patients are highly suspected of having isolated HH. However, the three patients are currently in prepuberty and need further follow-up.

Accordingly, *SOX2* mutations have broad phenotypic spectrum, from completely normal to severe ocular malformations and growth retardation, and most mutations are *de novo*. *SOX2* may cooperate with other HH pathogenic genes to cause non-syndromic HH. However, this study is a clinical observation with a limited sample size, and more studies are needed to confirm the mechanism of *SOX2* in non-syndromic HH.

Declaration of patient consent

The authors certify that they have obtained all the three patients' parents consent forms. Their parents understand that their children's names and initials will not be published and due efforts will be made to conceal their identity, but anonymity can't be guaranteed.

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Conflicts of interest

None.

References

1. Kelberman D, de Castro SC, Huang S, Crolla JA, Palmer R, Gregory JW, *et al*. SOX2 plays a critical role in the pituitary, forebrain, and eye during human embryonic development. J Clin Endocrinol Metab 2008;93:1865–1873. doi: 10.1210/jc.2007-2337.
2. Shima H, Ishii A, Wada Y, Kizawa J, Yokoi T, Azuma N, *et al*. SOX2 nonsense mutation in a patient clinically diagnosed with non-syndromic hypogonadotropic hypogonadism. Endocr J 2017;64:813–817. doi: 10.1507/endocrj.EJ17-0078.
3. Suzuki J, Azuma N, Dateki S, Soneda S, Muroya K, Yamamoto Y, *et al*. Mutation spectrum and phenotypic variation in nine patients with SOX2 abnormalities. J Hum Genet 2014;59:353–356. doi: 10.1038/jhg.2014.34.
4. Wang Y, Gong C, Qin M, Liu Y, Tian Y. Clinical and genetic features of 64 young male paediatric patients with congenital hypogonadotropic hypogonadism. Clin Endocrinol (Oxf) 2017;87:757–766. doi: 10.1111/cen.13451.
5. Takagi M, Narumi S, Asakura Y, Muroya K, Hasegawa Y, Adachi M, *et al*. A novel mutation in SOX2 causes hypogonadotropic hypogonadism with mild ocular malformation. Horm Res Paediatr 2014;81:133–138. doi: 10.1159/000355279.
6. Schneider A, Bardakjian TM, Zhou J, Hughes N, Keep R, Dorsainville D, *et al*. Familial recurrence of SOX2 anophthalmia syndrome: phenotypically normal mother with two affected daughters. Am J Med Genet A 2008;146A:2794–2798. doi: 10.1002/ajmg.a.32384.
7. Stark Z, Storen R, Bennetts B, Savarirayan R, Jamieson RV. Isolated hypogonadotropic hypogonadism with SOX2 mutation and anophthalmia/microphthalmia in offspring. Eur J Hum Genet 2011;19:753–756. doi: 10.1038/ejhg.2011.11.
8. Boehm U, Bouloux PM, Dattani MT, de Roux N, Dodé C, Dunkel L, *et al*. Expert consensus document: European consensus statement on congenital hypogonadotropic hypogonadism–pathogenesis, diagnosis and treatment. Nat Rev Endocrinol 2015;11:547–564. doi: 10.1038/nrendo.2015.112.

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