SOX2 heterozygous mutation causes multiple extra-ocular phenotypes in boys

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Abstract

Background The *SOX2* gene is widely expressed in the eyes and the central nervous system. Heterozygous mutations could cause eye malformations and hypopituitarism, and serve as the causative gene for syndromic and non-syndromic hypogonadotropic hypogonadism (HH). Our study reports three children with chromosome 46, XY, SRY (+), but *SOX2* mutations.

Methods Three children visited our endocrine clinic because of micropenis and/or cryptorchidism. Clinical data were collected, and one took PANEL sequencing and the others for whole exome sequencing. Then we summarized characteristics of the patients and compared with those mentioned in literature.

Results Patient 1 manifested with micropenis, patient 2 with bilateral cryptorchidism and craniofacial deformities, both carrying the same reported *SOX2* gene mutation (T232N), and both mutations from mothers with delayed puberty only. Patient 3 showed micropenis, mental retardation and craniofacial deformities, and the child carried a spontaneous truncation mutation (Y110X) of the *SOX2* gene. This site has reported that a missense mutation caused adolescent adolescence without major eye signs.

All three patients carried another gene mutations that affected hypothalamic-pituitary function: Patient 1, *FGFR1*: c.238C>T/p.R80C (uncertain) from father; Patient 2, *CHD7*: c.2656C>T/p.R886W (pathogenic) de novo; Patient 3, *SEMA3A*: c.1432G> A/p.E478K (uncertain) from mother. None had major ocular malformations, and all showed genitourinary tract malformations. Two patients had craniofacial deformities, and one patient had muscle anomality and intellectual disability.

We summarized previous studies with *SOX2* gene mutations and it showed: 71.2% of mutations are de novo, all patients reported whose variants inherit from parents, 15.1% parents (including mother 11.0% and father 4.1%) show completely normal phenotypes, 4.1% (3/73) variants inherit from mother with germinal mosaicism. Except for major ocular malformations (91.1%), the most common phenotype is developmental delay/mental retardation (DD/MR), accounting for 40.7%, followed by brain anomely (BA), accounting for 28.5%, male genital abnormalities (GA) for 20.3%, non-syndromic HH accounted for 4.9%, the younger the patients visit the doctor, the more common the retardation are.

Conclusion

SOX2 mutations could cause a broad phenotype spectrum from completely normal to severe ocular malformations, retardation and most mutations are de novo. Except for major ocular malformations and retardation, GA/HH is another common symptom. GA/HH may be the only symptom, and *SOX2* may cooperate with another HH pathogenic genes to cause non-syndromic HH.

Introduction

The HMG-box transcription factor SOX2 (OMIM 184429) is most notably expressed in eye, placodes, forebrain and hypothalamo-pituitary, and involved in the early embryonic development [1,2]. Loss of

function mutations or deletions of *SOX2* could lead to uni- or bilateral anophthalmia/microphthalmia (A / M) as well as other related disorders like anophthalmia/esophageal-genital syndrome (AEG) [3,4]. More and more studies found that *SOX2* mutations could cause variable extra-ocular symptoms, including growth retardation, sensorineural hearing loss, mental retardation, intellectual disability, brain malformation, no pubertal signs and male genitourinary tract malformations (micropenis, cryptorchidism and hypospadias)[5,6]. Indeed, cases with *SOX2* pathogenic mutations but no or minor ocular symptoms have been reported less frequently.

SOX2 is a highly conserved gene located in 3q26.3-27.1. The single exon gene encodes a protein of 317 residues, which includes an N-terminal domain, a DNA-binding HMG (high-motility group) domain and a transcriptional activation domain in C-terminal [7]. At present, more than 100 cases of ophthalmology patients with A / M or other major ocular anomalies have been reported to have *SOX2* mutations, mainly truncating variants caused by nonsense or frameshift [8–12]. SOX2 plays a pivotal role in the development of hypothalamo-pituitary by transactivation of mutiple genes including *HESX1*, and its mutations can cause hypophysial hypoplasia, reduce gonadotropin secretion, thus causing genital tract abnormalities or no puberty [2,13]. Several studies found that *SOX2* heterozygous mutations cause typical signs of complete hypogonadism without major ocular malformations in men or women, say, isolated hypogonatropic hypogonadism (HH), but no another HH pathogenic gene was identified [10–11,14]. And no such Chinese patients were reported as well. Therefore, our study reported for the first time that three Chinese boys who referred due to micropenis and/or cryptorchidism, combined with craniofacial deformities or intellectual disability. The NGS found *SOX2* heterozygous mutations and another HH pathogenic genes were identified. It is speculated that *SOX2* and other HH pathogenic genes cooperate to cause symptoms.

Subjects And Methods

We report three cases who visited endocrinology clinic because of micropenis and/or cryptorchidism. All their phenotypes, family history, hormone levels and genetic results were collected. The characteristics of the cases were summarized and compared with those involved in the literature.

The detection method of hormones, hCG standard and prolonged stimulating test and LHRH stimulation test were referred to the published literature of our group[15–16].

NGS was completed in the Kangxu Company, patient 1 was taken panel sequencing including 167 genes involved in gonadal development, both patients 2 and 3 were carried out whole exome sequencing. DNA was extracted from peripheral blood leukocytes of patients and their parents, and the NEXTSEQ 500 sequencer (Illumina corporation, USA) was used to filter out all possible pathogenic missense, frameshift and splice site mutations. Design primers and Sanger sequencing were used to verify the mutations of samples from the same family. Mutations of MAF < 1% in East Asia people were presented here. And we checked the pathogenicity of the mutations according to ACMG.

The research protocol was approved by the Ethics Committee of the National Children's Medical Center and the Beijing Children's Hospital. Written informed consent was obtained from all participants.

Results

Clinical and genetic features of three patients

Patient 1

The male patient was 6 months old at first visit, he was born at full term, and the birth weight and length were unknown. He was born with micropenis, but his intelligence and growth development were normal. He had no special facial features, no abnormalities in heart, lungs and abdomen. The penis is 2.3 cm long and 1.2 cm in diameter. The urethral meatus is normal. The volume of bilateral testis is about 2 ml. The mother had a threatened miscarriage at two months of pregnancy, and she received progesterone treatment for one month. His father's growth spurt was 17 years old, and his mother began menarche at age of 14–15 years old, and he had no family history of infertility.

The karyotype was 46, XY, and *SRY* was positive. Hormone levels were as follows: AMH > 23 ng/ml, INHB 126.2 pg/ml, T 434 ng dl after hCG stimulation test. LHRH stimulating test was performed in our hospital at the age of 3.5 years old. Basic LH 1.89 IU/L, FSH 5.48 IU/L, T < 20 ng/dl, peak LH/FSH = 3.47/5.48 = 0.63 > 0.6, suggesting that the pituitary has a response. MRI showed that pituitary, olfactory bulb, olfactory tract and olfactory sulcus were normal. Given the age of the patient, HH was suspected. He took testosterone undecanoate to treat micropenis for one month, then the penis was 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, and fundus examination was normal. Later, a PANEL sequencing containing 167 gonads-associated genes was taken and found a pathogenic *SOX2* varient from mother: c.695C > A/p.T232N, and an unreported pathogenic *FGFR1* mutation from father: c.238C > T/p.R80C at the same time.

Patient 2

The male patient visited our clinic at the age of 2 years and 7 months. He was born at full term, with birth weight of 4 kg and a birth length of 51 cm. He was born with bilateral cryptorchidism, and had normal intelligence and growth development. Examination: eyes were normal; protruding ear, no inner cochlea, low nasal bridge, high arch bow, crooked mouth when crying, curved fifth finger, penetrating palm, but no abnormalities in heart, lungs and abdomen. The penis is 3 cm long and 1 cm in diameter. The urethral meatus is normal. The bilateral testis were located in the scrotum after surgery, and the volume was about 1 ml. No abnormalities during maternal pregnancy. The father was 172 cm, had a history of acrosomal enzyme deficiency, but the development was unknown. The mother was 170 cm, had menarche at the age of 15–16. She had one one elder sister aged 7 years, 123 cm tall, and had normal

growth and development. The grandparents were consanguineous marriage. The father failed to have an IVF due to infertility, but the patient and his sister were born naturally.

The karyotype was 46, XY, and SRY was positive. Basic sex hormone levels at first visit 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, T 25 ng/dl after hCG prolonged test, which suggested testicular dysplasia. Now the child is 3 years and 2 months old, with normal intelligence, normal hearing, smell and vision, and no abnormalities in fundus examination. Genetic testing revealed that the patient carried both *SOX2* mutation (c.695C > A/p.T232N, from mother, reported and pathogenic mutation) and *CHD7* mutation (c.2656C > T/p.R886W, de novo, unreported and pathogenic mutation).

Patient 3

The male patient went to hospital because of micropenis, bilateral cryptorchidism and poor intellectural development at 5 months. Examination: sluggish face, flat pillow, flat nose, left ptosis, poor muscle strength at the back and neck, high palatal arch, decreased muscle strength of the limbs, fetal fat pad of two fingers. The penis was 1 cm long and 0.6 cm in diameter. The urethral opening was normal. Bilateral testes were located in the scrotum, the volume was about 1 ml. The patient was the first child after three gestation (one of the twins), and he was conceived through ovulation induction and artificial pregnancy. And he was born at full term, with birth weight of 3.1 kg. He could turn over at 7 months old, when he was 2 years and 8 months, he just could walk with one's help, understand simple instructions, pronounce "Mom and Dad", speak unclearly, response slowly, and he received continuous rehabilitation training. At 6 months of pregnancy, the mother had a miscarriage due to abdominal pain, and the mother didn't pregnant in next 4 years. The second pregnancy was through ovulation, but the fetus stopped developing at 2 months of pregnancy. The father had congenital scoliosis, and the development of puberty was unknown. The mother was 160 cm tall, the menarche was 13 years old, and she had polycystic ovary syndrome.

The karyotype of the patient was 46, XY, and SRY was positive. Basic hormone levels at 5 months were as follows: LH 1.4 IU/L, FSH 7.9 IU/L, T < 20 ng/dl, T 116 ng / dl after hCG stimulation test, AMH > 23 ng/ml, INHB: 74.5 pg/ml, IGF-1: 50.9 ng/ml, Peak LH/FSH = 2.59/7.9 = 0.33 < 0.6, after LHRH stimulation test, which suggested the pituitary gland could have a response. Thyroid function, ACTH and cortisol were normal. Pituitary MRI showed that the pituitary gland was normal, and the olfactory bulb, olfactory tract, and olfactory groove developed normally. The boy received testosterone undecanoate treatment for micropenis. The child was 3 years old nowadays, but he couldn't walk steadily, catching up with mental and physical development, and is unstable. He was 88.3 cm tall, and 2 cm below the 3rd percentile of the growth curve. After treatment with testosterone undecanoate, the penis growed to 3.5 cm long and 1.1 cm in diameter. Hearing, smell and vision were normal, and no abnormalities were found on fundus examination. Genetic testing revealed that the patient harbored both *SOX2* mutation (c.330C >

A/p.Y110X, de novo, unreported and pathogenic mutation) and *SEMA3A* mutation (c.1432G > A/p.E478K, from mother, unreported, and clinical significance is uncertain).

In our single centre, more than 800 patients with 46, XY DSD received genetic tests, 118 patients were suspected with HH among these, and *SOX2* gene mutation accounted for 2.5% of the 118 patients. Patient 1 showed only micropenis, without any deformity and mental and physical development disorders, while patient 2 carrying the same *SOX2* gene mutation manifested with bilateral cryptorchidism and craniofacial deformities. Their mothers are carriers of *SOX2* mutation, and both only show delayed menarche. Patient 3 showed micropenis, bilateral cryptorchidism, mental retardation, eye and craniofacial deformities, see Tables 1, 2 and Fig. 1 for details. Patient 1 and 2 carry the same reported pathogenic site mutation (p.T232N) of the *SOX2* gene, which is located in the carboxy terminal transcription activation region, and Patient 3 carries a nonsense mutation (p.Y110X), Close to the HMG region, which has not been reported in the literature, but there are reports of the same site missense pathogenic mutation (p.Y110C). Intersting, we found that all three cases carry another gene varient that caused HH meanwhile, see Fig. 1 for the combined gene mutation.

Literature summary and comparison with our patients

In 2003, the *SOX2* gene mutation was first reported in patients with A/M deformities. Up to now, a total of 123 cases have been reported, including male (57/109, 52.3%) and female (52/109, 47.7%), some are not provided, visiting age ranges from fetus to 65 years old, 43.9% (25/57) male patients showed genital abnormalities, see supplementary table 1 and Table 3. Almost all patients choose to ophthalmology clinic, except for those patients without major eye deformities. While all three patients in our study are male, and show no major ocular symptoms.

Among those patients, 91.1% of patients have major ocular deformities. All patients reported whose variants inherit from parents, 15.1% parents (including mother 11.0% and father 4.1%) show completely normal phenotypes, 4.1% (3/73) variants inherit from mother with germinal mosaicism. Among the extraocular symptoms, the most common is developmental delay/mental retardation (DD/MR), accounting for 40.7%, followed by brain anomely (BA), accounting for 28.5%, motor development delay (MD) for 22%, male genital abnormalities (GA, including micropenis, cryptorchidism and hypospadias) for 20.3%, short stature (SS) for 17.1%, facial dysmorphism (FD) accounting for 12.2%, non-syndromic HH accounted for 4.9%, including three male patients (2.4%). Clinical and genetic features of all patients without major ocular deformities were listed in Table 4. The three patients in the study were mainly genital abnormalities, left ptosis, hypotonia, short stature, and intellectual impairment, but with no major ocular abnormalities. No seizures were found.

By analyzing all reported mutations of *SOX2*, mutational types include frameshift (39.4%), deletion (22%), nonsense (19.7%), missense (18.9%), 71.2% (53/73) of those are de novo, 15.1% (11/73) cases inherit from mother (11.0%) or father (4.1%) with completely normal phenotypes, 4.1% (3/73) variants inherit from mother with germinal mosaicism but normal phenotypes, and 5.5% (4/73) variants from their parents manifested with abnormal syndromes, including one farther with ocular deformity, one mother

with HH. And in our three patients, mutations of patients 2 and 3 are de novo, and mutation of patient 1 inherited from mother with normal phenotypes.

Discussion

The unilateral or bilateral A/M itself has a low prevalence rate. Among them, the detection rate of *SOX2* gene heterozygous mutations is about 10–20% [9], and some patients are accompanied by extra-ocular symptoms, including growth retardation, sensorineural hearing loss, intellectual impairment, corpus callosum dysplasia, pubertal dysplasia, male external genital malformation (micropenis, cryptorchidism and hypospadias) [36]. However, there are still few reports of patients with the gene variants who have no or minor eye symptoms.

1.
Phenotypes caused by SOX2 **mutations range from normal to severe ocular symptoms**

Totally 91.1% patients show A/M or severe coloboma in all cases reported with *SOX2* mutatitons. All patients reported whose variants inherit from parents, 15.1% parents (including mother 11.0% and father 4.1%) show completely normal phenotypes, 4.1% (3/73) variants inherit from mother with germinal mosaicism, both children in 3 families [21,23,24] have major ocular deformities, while their mothers manifest normal phenotypes.

And in Table 4, we present 11 cases with *SOX2* mutations but without major ocular deformities. Of our more than 500 genetically positive 46, XY DSD cases in our single centre, only 3 patients (0.6‰) were reported in the study, suggesting that the incidence of *SOX2* gene mutations is lower in patients without major ocular deformities. In our study, we reports 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 of *SOX2* gene (c.695C > A / p.T232N) transmitted from the mother, but the phenotypes are very different. Patient 1 shows merely micropenis, while patient 2 manifested bilateral cryptorchidism, facial deformities and crooked mouth crying syndrome, but both had normal vision and no ocular deformities, and their mothers have delayed menarche and no other phenotypes. Crooked mouth crying syndrome could manifest as deformities of eyes and ears and congenital heart disease, but it often cause by a minor deletion of chromosome 22q11, therefore, phenotypes of patient 2 couldn't be explained by the syndrome. Previous study report that 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 also showed ocular defects, arachnoid cysts and penoscrotal hypospadias, but no follow-up description of pubertal development and fertility [37].

In another family report containing a frameshift mutation in the *SOX2* gene (p.G280Afs91X), the mother was diagnosed with IHH 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 rears one son and one daughter, the son shows anophthalmia, and the daughter has unilateral microphthalmia

deformity. All of these suggest that patients with *SOX2* mutations have a broad phenotype spectrum, and there is no obvious correlation between genotypes and phenotypes [9,38].

2.
SOX2 **may cooperate with other pathogenic genes associated with hypothalamic-pituitary axis to cause HH**

Heterozygous *SOX2* mutations in human patients commonly cause pituitary hypoplasia on imaging, usually leading to low concentrations of LH and FSH (that is, typical HH), or GH deficiency and short stature in some conditions. However, HH was also observed in patients without pituitary hypoplasia, further study showed that *SOX2* mutations could reduce GnRH numbers and misdirect axonal projections, as evidenced by the phenomenon that HH patients carrying *SOX2* mutations could respond to GnRH stimulation. Our previous study and others showed multiple gene defects might synergize to cause a more severe HH phenotype in at least 20% of cases.

Heterozygous mutations in the *SOX2* gene could lead to syndromic HH with A/M/coloboma [4,5,9]. Since 2003, only 6 cases (4.9%) of patients with non-syndromic HH (except for absense of puberty, some patients also show micropenis and/or cryptorchidism) have been reported [8,11,14,30,38–39], while micropenis and cyptorchidism are the main clues of HH during childhood. The 3 patients in our study are currently young and need further follow-up. However, considering the symptoms, signs and genetic results, they are highly suspected diagnosis of IHH (including patient 2 with crooked mouth crying syndrome), which suggests that phenotypes causing by *SOX2* mutations can be various.

However, previous reports have not detected or only detected a small part of the HH pathogenic genes, therefore, only the *SOX2* gene mutation was found to be the causative gene for non-syndromic HH. According to previous reports, the *SOX2* gene heterozygous pathogenic mutations could cause related malformations in the study [40], but the specific mechanism and whether it may be synergistic with another HH gene still need further study. Our study provided clues for the different phenotypes couldn't be ruled out as the result of oligogenic genetic architecture.

Patient 2 carried both *SOX2* and *CHD7* gene mutation simultaneously, T was still low after the hCG prolonged test, and the INHB level was low, suggesting that the patient may had testicular Leydig cells and Sertoli cells dysplasia. One study has found that *SOX2* and *CHD7* could form a complex, and cooperatively activate downstream genes (such as Notch and Shh pathway genes) to participate in the development and maturation of testicular cells [41]. It is speculated that the *SOX2* and *CHD7* mutations in this patient may aggravate the impact of a single gene mutation on testicular function, which needs to be confirmed by further functional studies.

Patient 3 carried a new nonsense mutation (p.Y110X) of *SOX2* gene, and he showed micropenis, cyptorchidism and mental retardation but normal vision. In 2014, Takagi et al. reported a missense mutation (p.Y110C) of the *SOX2* gene at this site, the patient presented with micropenis and no pubertal development at the age of 20, he began to appear generalized seizures at the age of 3, and received right

retinal detachment surgery at the age of 14 years old. Y110 is a critical amino acid near the DNA-binding HMG region. Mutation attenuates the activation of the downstream target gene *HESX1* [30]. While the patient in our study occurred a nonsense mutation at this site, and it's speculated that the mutation would have a greater impact on target gene activation and subsequent gonadotropin levels.

Conclusion

SOX2 mutations could cause a broad phenotype spectrum from completely normal to severe ocular malformations, retardation and most are de novo. Except for major ocular malformations and retardation, GA/HH is another common symptom. GA/HH may be the only symptom, and SOX2 may cooperate with another HH pathogenic genes to cause non-syndromic HH.

Abbreviations

A/M:anophthalmia/microphthalmia; HMG:high-motility group; HH:hypogonatropic hypogonadism; SOX2: SRY-related HMG box 2; CHD7:chromodomain helicase DNA-binding protein 7; SEMA3A:semaphorin 3A; FGFR1:fibroblast growth factor receptor 1; NGS:next-generation sequencing; DSD:disorders of sex diseases.

Declarations

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Authors' contributions

Yi Wang collected, analysed and wrote the paper, Lijun Fan, Xiaoya Ren, Yanning Song and Beibei Zhang helped collect data, Chunxiu Gong revised the paper.

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Availability of data and materials

Please contact author for data requests.

Ethics approval and consent to participate

The research protocol was approved by the ethics committee of Beijing Children's Hospital, Capital Medical University.

Consent for publication

Written informed consent was received from all patients or legal guardians.

Competing interests

The authors declares that they have no competing interests.

References

1. Kelberman D, Rizzoti K, Lovell-Badge R, Robinson IC, Dattani MT. Genetic regulation of pituitary gland development in human and mouse. Endocr Rev. 2009;30(7):790-829.
2. 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; 95(5):1865-73.
3. Williamson KA, Hever AM, Rainger J, Rogers RC, Magee A, Fiedler Z, et al. Mutations in *SOX2* cause anophthalmia-esophageal-genital (AEG) syndrome. Hum Mol Genet. 2006; 15(9):1413-22.
4. Ragge NK, Lorenz B, Schneider A, Bushby K, de Sanctis L, de Sanctis U, et al. *SOX2* anophthalmia syndrome. Am J Med Genet A. 2005; 135(1):1-7.
5. Hagstrom SA, Pauer GJ, Reid J, Simpson E, Crowe S, Maumenee IH, et al. *SOX2* mutation causes anophthalmia, hearing loss, and brain anomalies. Am J Med Genet A. 2005; 138A(2):95-8.
6. Kelberman D. Mutations within *Sox2/SOX2* are associated with abnormalities in the hypothalamo-pituitary-gonadal axis in mice and humans. J Clin Invest. 2006; 116(9):2442-55.
7. Mandalos N, Saridaki M, Harper JL, Kotsoni A, Yang P, Economides AN, et al. Application of a novel strategy of engineering conditional alleles to a single exon gene, *Sox2*. PLoS One. 2012; 7(9):e45768.
8. Schneider A, Bardakjian T, Reis LM, Tyler RC, Semina EV. Novel *SOX2* mutations and genotype-phenotype correlation in anophthalmia and microphthalmia. Am J Med Genet A. 2009; 149A(12):2706-15.
9. Chassaing N, Causse A, Vigouroux A, Delahaye A, Alessandri JL, Boespflug-Tanguy O, et al. Molecular findings and clinical data in a cohort of 150 patients with anophthalmia/microphthalmia. Clin Genet. 2014;86(4):326-34.
10. 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(6):353-6.
11. Shima H, Ishii A, Wada Y, Kizawa J, Yokoi T, Azum N, et al. *SOX2* nonsense mutation in a patient clinically diagnosed with non-syndromic hypogonadotropic hypogonadism. Endocr J. 2017; 64(8): 813-7.
12. Dennert N, Engels H, Cremer K, Becker J, Wohlleber E, et al. De novo microdeletions and point mutations affecting *SOX2* in three individuals with intellectual disability but without major eye malformations. Am J Med Genet A. 2017; 173:435-43.

13. Jayakody SA, Andoniadou CL, Gaston-Massuet C, Signore M, Cariboni A, Bouloux PM, et al. *SOX2* regulates the hypothalamic-pituitary axis at multiple levels. J Clin Invest. 2012; 122(10):3635-46.
14. Sato N, Kamachi Y, Kondoh H, Shima Y, Morohashi K, Horikawa R, et al.Hypogonadotropic hypogonadism in an adult female with a heterozygous hypomorphic mutation of SOX2. Eur J Endocrinol. 2007; 156(2):167-71.
15. 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(6):757-66.
16. Gong CX, Liu Y, Qin M, Wu D, Wang X. Pulsatile GnRH is superior to hCG in therapeutic efficacy in adolescent boys with hypogonadotropic hypogonadodism. J Clin Endocrinol Metab. 2015; 100(7): 2793-9.
17. Fantes J, Ragge NK, Lynch SA, McGill NI, Collin JR, Howard-Peebles PN, et al. Mutations in SOX2 cause anophthalmia. Nat Genet. 2003; 33(4):461-3.
18. Zenteno JC, Perez-Cano HJ, Aguinaga M. Anophthalmia-esophageal atresia syndrome caused by an SOX2 gene deletion in monozygotic twin brothers with markedly discordant phenotypes. Am J Med Genet A. 2006; 140(18):1899-903.
19. Faivre L, Williamson KA, Faber V, Laurent N, Grimaldi M, Thauvin-Robinet C, et al. Recurrence of SOX2 anophthalmia syndrome with gonosomal mosaicism in a phenotypically normal mother. Am J Med Genet A. 2006; 140(6):636-9.
20. Bakrania P, Robinson DO, Bunyan DJ, Salt A, Martin A, Crolla JA, et al. SOX2 anophthalmia syndrome: 12 new cases demonstrating broader phenotype and high frequency of large gene deletions. Br J Ophthalmol. 2007; 91(11):1471-6.
21. Chassaing N , Gilbert-Dussardier B, Nicot F, Fermeaux V, Encha-Razavi F, Fiorenza M, et al. Germinal mosaicism and familial recurrence of a SOX2 mutation with highly variable phenotypic expression extending from AEG syndrome to absence of ocular involvement. Am J Med Genet A. 2007;143A(3):289-91.
22. Wang PF, Liang XL, Yi JH, Zhang QJ.Novel SOX2 mutation associated with ocular coloboma in a Chinese family. Arch Ophthalmol. 2008;126(5):709-13.
23. Zhou J, Kherani F, Bardakjian TM, Katowitz J, Hughes N, Schimmenti LA. Identification of novel mutations and sequence variants in the SOX2 and CHX10 genes in patients with anophthalmia/microphthalmia. Mol Vis. 2008; 14:583-92.
24. 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(21):2794-8.
25. 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(7):753-6.
26. Gonzalez-Rodriguez J, Pelcastre EL, Tovilla-Canales JL, Garcia-Ortiz JE, Amato-Almanza M, Villanueva-Mendoza C, et al. Mutational screening of CHX10, GDF6, OTX2, RAX and SOX2 genes in

50 unrelated microphthalmiae anophthalmiae coloboma (MAC) spectrum cases. Br J Ophthalmol. 2010; 94(8):1100-4.

27. Alatzoglou KS, Andoniadou CL, Kelberman D, Buchanan CR, Crolla J, Arriazu MC, et al. SOX2 haploinsufficiency is associated with slow progressing hypothalamo-pituitary tumours. Hum Mutat. 2011; 32(12):1376-80.
28. Gerth-Kahlert C, Williamson K, Ansari M, Rainger JK, Hingst V, Zimmermann T, et al. Clinical and mutation analysis of 51 probands with anophthalmia and/or severe microphthalmia from a single center. Mol Genet Genom Med. 2013; 1(1):15-31.
29. Schilter KF, Reis LM, Schneider A, Bardakjian TM, Abdul-Rahman O, Kozel, BA, et al. Whole-genome copy number variation analysis in anophthalmia and microphthalmia. Clin Genet. 2013; 84(5): 473-81.
30. 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(2):133-8.
31. Macchiaroli A, Kelberman D, Simona Auriemma R, Drury S, Islam L, Giangiobbe S, et al. A novel heterozygous SOX2 mutation causing congenital bilateral anophthalmia, hypogonadotropic hypogonadism and growth hormone deficiency. Gene. 2014; 534(2):282-5.
32. Mauri L, Franzoni A, Scarcello M, Sala S, Garavelli L, Modugno A, et al. SOX2, OTX2 and PAX6 analysis in subjects with anophthalmia and microphthalmia. Eur J Med Genet. 2015;58(2):66-70.
33. Ammar THA, Ismail S, Al-Motaal Mansour OA , El-Shafey MM, Doghish AS , Kamal AM, et al. Genetic analysis of SOX2 and VSX2 genes in 27 Egyptian families with anophthalmia and microphthalmia. Ophthalmic Genet. 2017;38(5):498-500.
34. 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(8):813-7.
35. Errichiello E, Gorgone C, Giuliano L, Iadarola B, Cosentino E, Rossato M, et al. SOX2: Not always eye malformations. Severe genital but no major ocular anomalies in a female patient with the recurrent c.70del20 variant. Eur J Med Genet. 2018;61(6):335-40.
36. Leiden University Medical Center. Leiden Open Variation Database. MRC Human Genetics Unit LOVD at MRC IGMM. Leiden University Medical Center Web site. http://lsdb.hgu.mrc.ac.uk/home.php. Acessed June 7, 2017.
37. Wang PF, Liang XL, Yi JH, Zhang QJ. Novel *SOX2* mutation associated with ocular coloboma in a Chinese family. Arch Ophthalmol. 2008; 126(5):709-13.
38. 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(7):753-6.
39. Sato N, Kamachi Y, Kondoh H, Shima Y, Morohashi K, Horikawa R, et al. Hypogonadotropic hypogonadism in an adult female with a heterozygous

40. hypomorphic mutation of *SOX*2. Eur J Endocrinol.2007; 156(2):167-71.
41. Topaloglu AK, Kotan LD. Genetics of hypogonadotropic hypogonadism. Endocr Dev. 2016; 29:36-49.
42. Engelen E, Akinci U, Bryne JC, Hou J, Gontan C, Moen M, et al. *Sox2* cooperates with *Chd7* to regulate genes that are mutated in human syndromes. Net Genet. 2011; 43(6): 607-11.

Tables

Due to technical limitations, Tables 1-3 are provided in the Supplementary Files section.

Figures

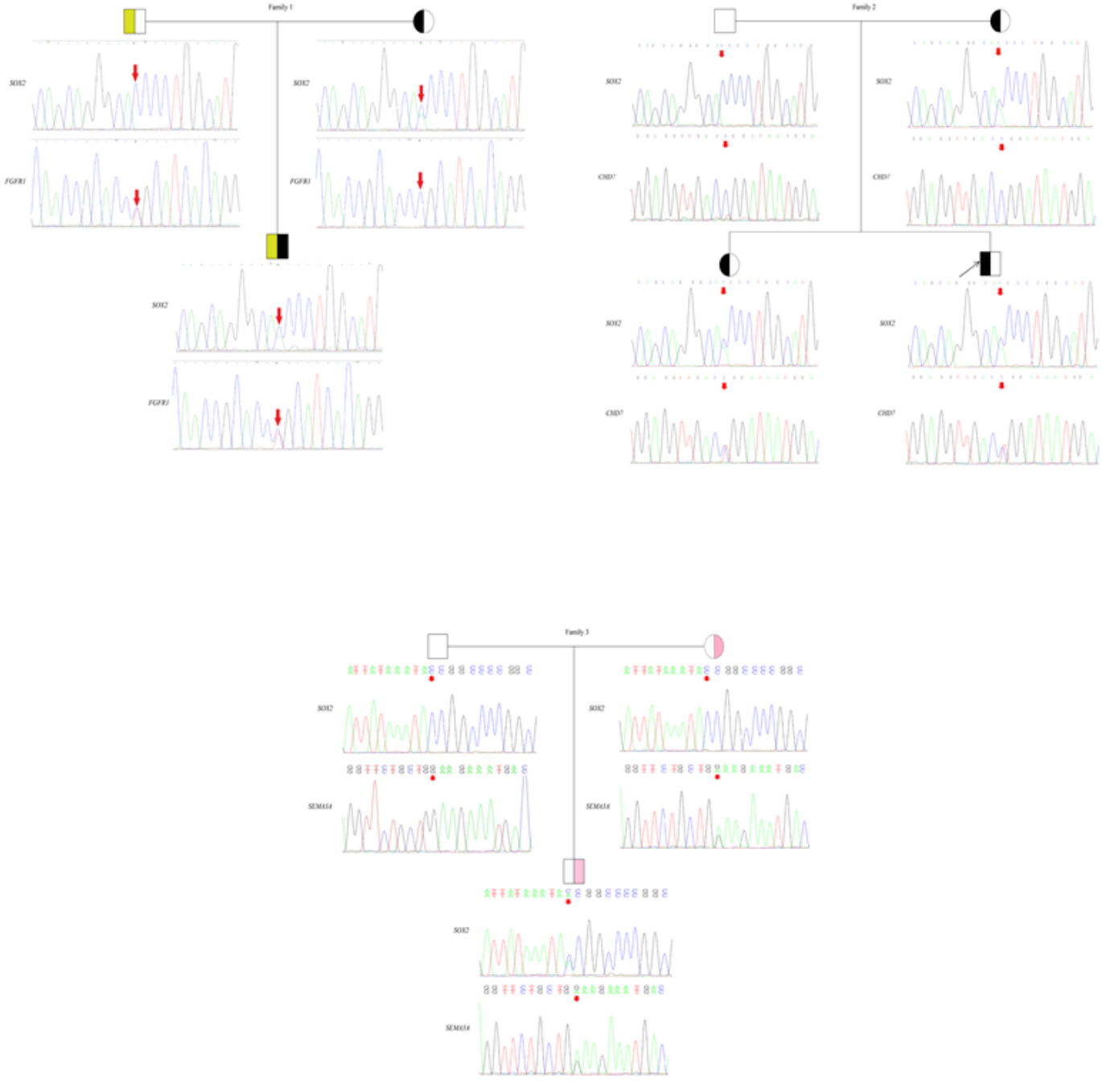

Figure 1

SOX2 and another gene mutations carrying by the three patients

Supplementary Files

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- Supplementarytable1.doc