Journal of Genetics, Vol. 97, No. 4, September 2018, pp. 1007–1011
https://doi.org/10.1007/s12041-018-0970-4

RESEARCH NOTE

A novel deletion mutation of the *SOX2* gene in a child of Chinese origin with congenital bilateral anophthalmia and sensorineural hearing loss

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Received 31 July 2017; revised 17 November 2017; accepted 16 January 2018; published online 18 August 2018

Abstract. Congenital anophthalmia is a rare eye anomaly which lacks a recognizable eye in the orbit. It can be isolated (nonsyndromic) or be observed as a sign of other diseases (syndromic). A Chinese infant was born with bilateral anophthalmia and palpebral fissure closures. Ocular and systemic examinations were performed, and genomic DNA was prepared from peripheral leukocytes. The coding exons and the adjacent intrinsic sequence of *SOX2* were analysed by Sanger sequencing. A c.70_89del (p. Asn24ArgfsX65; rs398123693) mutation in *SOX2* was identified in the Chinese infant with bilateral clinical anophthalmia and sensorineural hearing loss. This mutation was not detected in the unaffected parents and 150 unaffected control individuals. Mutation in *SOX2* is associated with bilateral clinical anophthalmia and probably with other anomalies in the Chinese infant. Until now hearing loss has not been reported in individuals with *SOX2* mutation. The results remind us that clinical anophthalmia may be accompanied by sensorineural hearing loss and may be associated with *SOX2* mutation, and it will contribute to improving diagnosis and patient care. Given that children with anophthalmia already have reduced sight, it seems worthwhile to make a point of careful vigilance on hearing for all such patients.

Keywords. congenital anophthalmia; sensorineural hearing loss; genomic DNA; *SOX2* gene; Sanger sequencing.

Introduction

Congenital anophthalmia is a rare eye anomaly in which a recognizable eye is not found in the orbit. This condition has a prevalence rate between 0.3 and 0.6 per 10,000 births (Tucker *et al*. 1996). This defect can be isolated (nonsyndromic) or observed in combination with other congenital abnormalities (syndromic) (Wang *et al*. 2008). The aetiology of congenital anophthalmia is diverse and includes external gestational factors and primary genetic defects. Heritable causes of anophthalmia involve chromosome abnormalities (autosomal dominant, autosomal recessive, or X-linked inheritance) and syndromic or nonsyndromic single-gene disorders. The *SOX2* gene, which is located at chromosome 3q26.3-q27, encodes a 317 amino acid protein that belongs to the high-mobility group (HMG) DNA-binding protein family. Because *SOX2* is a transcription factor that is specifically expressed in the developing eye and nervous system, and plays a regulatory role in lens development, the gene may be associated with severe structural eye defects (Fantes *et al*. 2003). Indeed, *SOX2* mutations appear to represent the most common causes of anophthalmia and are found in up to 10% of patients with microphthalmia or anophthalmia (Bakrania *et al*. 2007). Previously reported cases of anophthalmia with extraocular abnormalities (not including hearing loss) were associated with *SOX2* partial gene deletions. In this study, a 20 bp *de novo* deletion (c.70_89del) in *SOX2* was identified in an infant of Chinese origin who exhibited bilateral anophthalmia and sensorineural hearing loss.

Materials and methods

The proband is the first child of healthy, nonconsanguineous parents of Chinese origin, and there was no prenatal exposure to potential chemical or biological teratogens. The proband was born with bilateral anophthalmia and palpebral fissure closures (figure 1). Systemic examinations were performed after the proband

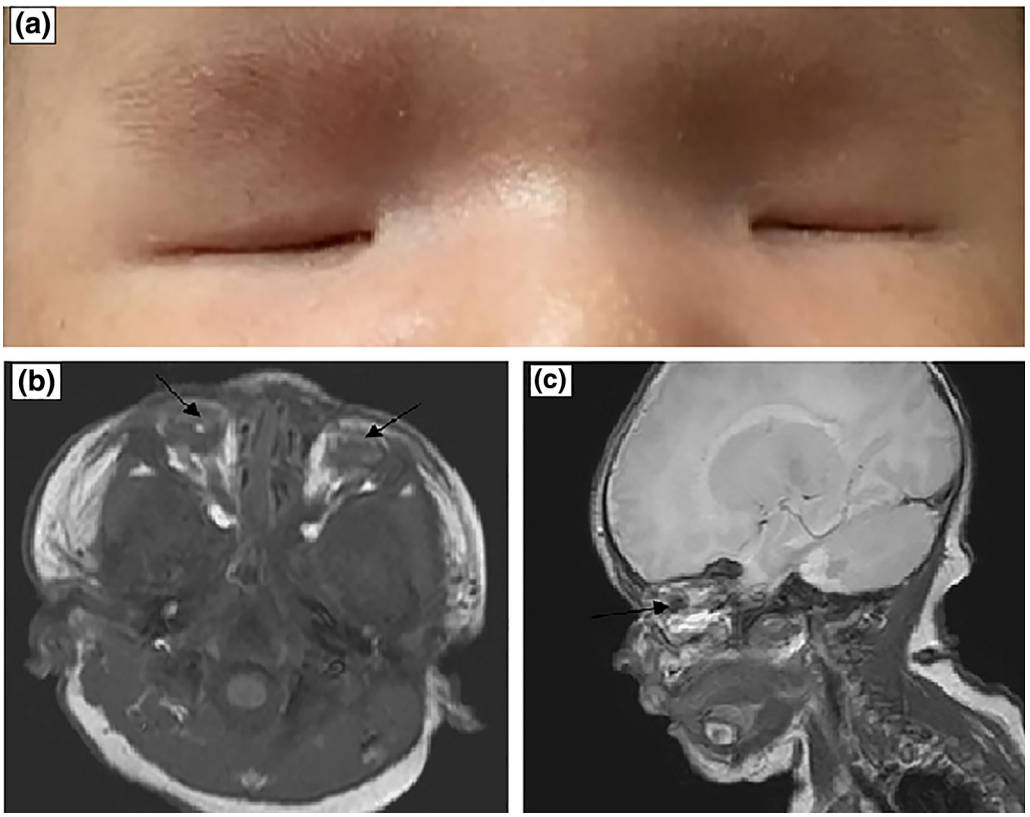

Figure 1. External photograph and brain MRI of the patient. (a) Bilateral clinical anophthalmia, complete fusion of the eyelid and hypoplastic sockets. (b) Axial T1-weighted image showing bilateral anophthalmia (arrows) and hypoplastic orbits. (c) Sagittal high-resolution T1-weighted image showing the anophthalmia (arrows).

was admitted to our hospital. In accordance with the Declaration of Helsinki, informed consent was obtained from the participating individuals before the study. This study was approved by the Institutional Review Board of the Lu Zhou Ophthalmic Center.

Peripheral blood samples were collected from the patient and her parents, as well as from 100 normal adults. Genomic DNA was prepared from peripheral blood leukocytes using standard techniques. The *SOX2* coding sequence was amplified using standard polymerase chain reaction (PCR) methodologies (primers and conditions are available on request), and the purified PCR products were sequenced in both directions using Big Dye Terminator v3.1 (Applied Biosystems, Fpster City, USA) with a 3730XL DNA Analyzer (Applied Biosystems). The sequencing files were examined manually using Polyphred software.

Results

The child of Chinese origin was born with bilateral anophthalmia and palpebral fissure closures (figure 1a). The head, bilateral eyebrows and eyelashes were normal, but the conjunctiva and the eyeball were not observed. Hearing tests revealed severe bilateral sensorineural hearing loss. Orbital and brain magnetic resonance imaging (MRI) demonstrated complete absence of bilateral eye globes but did not reveal any intracranial malformation (figure 1, b&c). Systemic examination did not reveal any other abnormalities.

DNA sequencing of *SOX2* genes in the patient revealed that in one *SOX2* allele, a deletion of 20 bp, i.e. from nucleotide 70 to nucleotide 89 (c.70_89del; figure 2) near the 5′ end of the gene was found. This deletion results in a frame-shift mutation upstream of the HMG box-coding region and predicts the introduction of a premature termination signal 65 codons downstream (p. Asn24ArgfsX65). The predicted truncated SOX2 protein consists of only 87 amino acids instead of the 317 residues of the normal protein, which would result in the removal of both the HMG and the C-terminal transactivation domains. This alteration was not identified in her normal parents or in 100 unrelated DNA control samples from individuals without known eye diseases. Standard parentage testing using informative microsatellites on chromosomes 4q, 5q, 6p, 12p, 12q, 15q and Xq was consistent with both parents of the patient being the biological parents ($P>0.995$). We conclude that this sequence change is a *de novo* mutation that caused the phenotype in this patient.

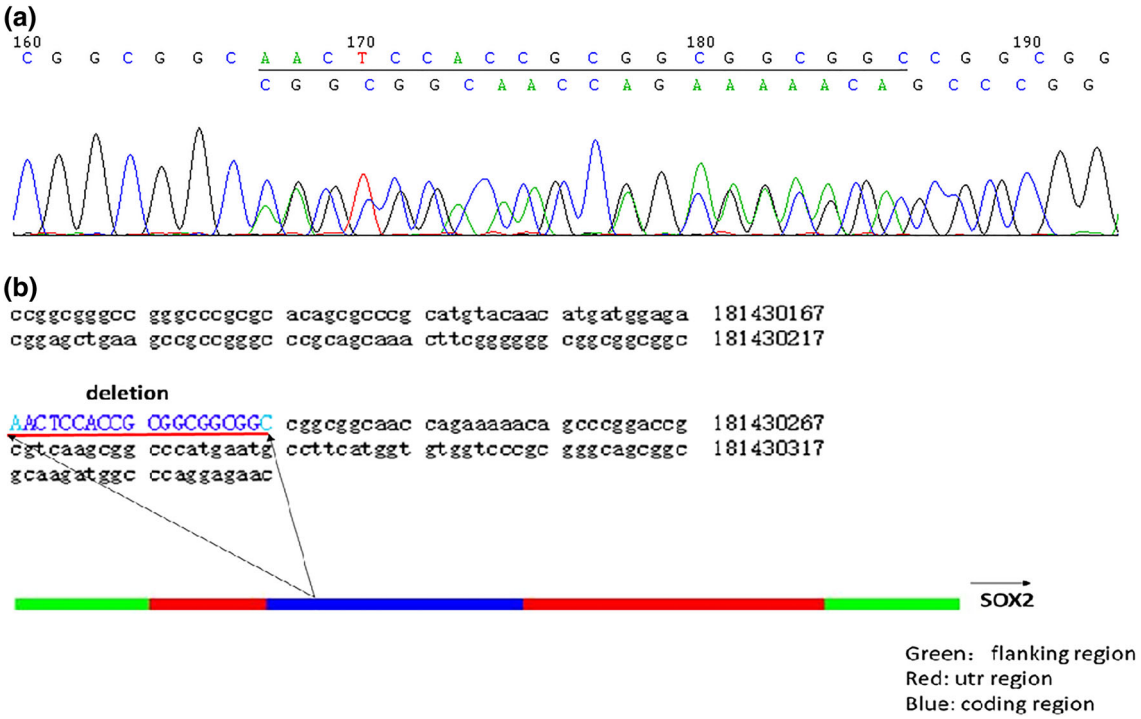

Figure 2. A 20-bp deletion (c.70_89del) of the *SOX2* gene in a child of Chinese origin with bilateral anophthalmia. (a) The upper nucleotide sequence corresponds to the wild-type *SOX2* allele in which the underline indicates the 20 nucleotides deleted in DNA from the patient (lower sequence). (b) The flanking regions are shown as green fragments, the UTRs are shown as red fragments, and the coding region is shown as a blue fragment. The 20-bp deletion (c.70_89del) of the *SOX2* gene comprises the red underlined nucleotides.

Discussion

Eye development involves five broad, temporally overlapping processes: optic vesicle outgrowth, optic cup/lens induction, optic fissure closure, chamber formation and functional maturation. The SOX2 protein, which acts as a transcription factor, is expressed mostly in all stages of eye development (Fantes *et al.* 2003), and interruption of any of the above processes could result in an absent or a small, structurally abnormal eye. In previous studies, complete absence of the *SOX2* gene and *SOX2* gene loss-of-function mutations were shown to lead to similar clinical manifestations. Therefore, the mechanism by which *SOX2* gene defects cause eye abnormalities appears to be associated with *SOX2* haploinsufficiency (Ragge *et al.* 2005). Mutations in the *SOX2* gene found in patients with congenital microphthalmia and anophthalmia include deletions, nonsense mutations, frame-shift mutations, missense mutations and mutations of the 3′ untranslated region (UTR) (Bakrania *et al.* 2007). The *SOX2* gene has only one exon, and all these mutations may result in the loss of SOX2 protein function, which may be the underlying cause of *SOX2* gene-related defects. Under this assumption, *SOX2* gene deletion and point mutations can cause similar clinical manifestations. Another issue of concern is that it is impossible to speculate how a specific *SOX2* gene mutation can lead to eye abnormalities. The clinical manifestations are related to the initial period of *SOX2* expression during eye development, and earlier occurrence leads to more serious clinical manifestations (Ragge *et al.* 2005). If the initial lesion occurs during formation of the optic vesicle, it may result in failure of the optic vesicle development, resulting in absence of eye, and formation of the nerve will not occur.

To date, the *SOX2* gene c.70_89del mutation, which results in a severe phenotype characterized by bilateral anophthalmia/microphthalmia and other anomalies, has been reported in 14 patients from 13 unrelated pedigrees (table 1). Previous studies have reported other anomalies including brain malformations (Zenteno *et al.* 2005), oesophageal atresia, absence of visible stomach (Schneider *et al.* 2009), horseshoe kidney (Bakrania *et al.* 2007), absence of pubertal development with hypogonadotropic hypogonadism, hamartoma of the tuber cinereum, micropenis, cryptorchidism, foreskin adhesion (Schneider *et al.* 2009), umbilical hernia (Reis *et al.* 2010), cavum vergae anomalies (Williamson and Fitz-Patrick 2014), and dental anomalies (Chacon-Camacho *et al.* 2015), but sensorineural hearing loss has not been reported. It is not clear how these phenotypes are related to the expression and function of *SOX2*. Given this variability, even among patients with the same mutation, it is likely that background genetic or environmental variations have significant influences. To the best of our knowledge, the

Table 1. Clinical findings in patients with the recurrent c.70_89del mutation in *SOX2*.

Reported case	Race	Right eye	Left eye	Other anomalies	Reference
1	Mexican	AN	AN	Brain malformations	Zenteno *et al.* (2005)
2 (twin A)	Mexican	NOR	AN	Oesophageal atresia	Schneider *et al.* (2009)
3 (twin B)	Mexican	NOR	NOR	Absence of visible stomach	Schneider *et al.* (2009)
4	Englishman	AN	CA, COL, GL	Oesophageal atresia and horseshoe kidney	Bakrania *et al.* (2007)
5	Englishman	AN	AN	None	Bakrania *et al.* (2007)
6	Englishman	AN	AN	Absence of pubertal development with HH	Schneider *et al.* (2009)
7	Hispanic	MI	MI	Hamartoma of tuber cinereum	Schneider *et al.* (2009)
8	Hispanic	AN	AN	Micropenis and cryptorchidism	Schneider *et al.* (2009)
9	Hispanic	AN	AN	Foreskin adhesion	Schneider *et al.* (2009)
10	American	MI	NOR	Micropenis, cryptorchidism and umbilical hernia	Reis *et al.* (2010)
11	European	AN	NOR	Cavum verge anomalies	Williamson and FitzPatrick (2014)
12	European	NOR	AN	None	Williamson and FitzPatrick (2014)
13	Mexican	MI	AN	Dental anomalies	Chacon-Camacho *et al.* (2015)
14	Chinese	AN	AN	Hearing loss	This study

AN, anophthalmia; MI, microphthalmia; CA, cataract; COL, coloboma; GL, glaucoma; NOR, normal; HH, hypogonadotropic hypogonadism.

case described in this study represents the first occurrence of the c.70_89del mutation in a child of Chinese origin. Moreover, our report describes the first case of bilateral clinical anophthalmia and sensorineural hearing loss associated with the c.70_89del mutation. The c.70_89del mutation appears to be the major recurrent mutation in *SOX2* (Fantes *et al.* 2003). Two similar types of deletions, a 17-bp deletion at the same nucleotide position and a 23-bp deletion in the preceding codon, have been reported from different families (Ragge *et al.* 2005). Due to the presence of a GGCGGC repeated sequence flanking the region, slipped-strand mispairing has been considered the likely molecular mechanism for this recurrent mutation. In this anomaly, one DNA strand of a repeat may be misaligned by chance with the downstream repeat of the complementary strand (Zenteno *et al.* 2005).

Recent studies have demonstrated heterozygous loss-of-function mutations in *SOX2* in 10–15% of patients with bilateral anophthalmia (Fantes *et al.* 2003). Although most mutations occur *de novo* in the proband, Wang *et al.* (2008) reported a case of congenital coloboma of the eyeball in which the proband had a normal-sized eye and his father had the same *SOX2* gene mutation. In addition, Faivre *et al.* (2006) and Schneider *et al.* (2008) reported the 'pseudo-autosomal recessive inheritance' phenomenon, whereby the unaffected parents of probands may have a mosaic germline mutation. In the two families studied, the siblings exhibited bilateral anophthalmia; the parents were normal, but the studies confirmed that the mothers harboured a *SOX2* gene mutation as a germline chimera. We conclude that the prevalence risk of proband siblings depends on parental genotypes, but if proband's parents have germline chimeras, the prevalence risk for the siblings may be high. In our study, the parents of the patient had a normal phenotype and genotype, and the prevalence risk for proband siblings was very less. However, if the family wants to have another child, anophthalmia-related genetic counselling and an antenatal examination should be performed.

In summary, we have described a patient with a *de novo* c.70_89del (p. Asn24ArgfsX65) *SOX2* mutation, bilateral anophthalmia, palpebral fissure closures and bilateral sensorineural hearing loss. Although sensorineural hearing loss is not commonly reported in the *SOX2* anophthalmia syndrome, we suggest that a hearing examination should be performed in patients with *SOX2* mutations. Given that children with anophthalmia already have reduced sight, it seems worthwhile to execute careful vigilance with regard to hearing in all such patients.

Acknowledgements

We would like to thank the family members who participated in this study. This work was supported by the National Natural Science Foundation of China (no. 81570841) and the Southwest Medical University Affiliated Hospital Foundation (no. 16004).

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Corresponding editor: KUNAL RAY