Novel SOX2 Mutation Associated With Ocular Coloboma in a Chinese Family

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Objectives: To report a novel SOX2 (OMIM 184429) mutation in a Chinese family and to describe its ocular and extraocular clinical features.

Methods: Ocular and systemic examinations were performed, and genomic DNA was prepared from peripheral leukocytes. The coding exons and the adjacent intronic sequence of SOX2 were analyzed by cycle sequencing.

Results: A novel heterozygous c.695C>A (p.Thr232Asn) mutation in SOX2 was identified in a Chinese family in which both the father and the son had iris and chorioretinal uveal colobomas. In addition, cataracts were noted in the father but not in the son. Other anomalies were not found in the father but were present in the son, including brain arachnoid cyst, microcornea, retrolubular colobomatous orbital cyst, and penoscrotal hypospadias. This mutation was not detected in the unaffected mother and 103 unaffected control individuals.

Conclusions: Mutation in SOX2 is associated with typical ocular coloboma and probably other anomalies in this Chinese family. Arachnoid cyst has not been reported in individuals with the SOX2 mutation.

Clinical Relevance: The results remind us that ocular coloboma may be accompanied by arachnoid cyst and may be associated with SOX2 mutation, which will be helpful for improving diagnosis and patient care.

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Ocular coloboma is a rare eye malformation caused by failure of the optic fissure to close.\(^1,2\) It may involve various parts of the eye including the iris, choroid, retina, optic nerve, and ciliary body. Classic defects are partial absence of the inferior quadrant of the iris, choroid, and retina. In addition, cataract, microphthalmia, and anophthalmia are frequently associated with ocular coloboma.\(^3\) Colobomatous cyst is a subset of ocular coloboma in which a retrolubular cyst is present in addition to iris and choroid defects.\(^3\) Ocular coloboma may be manifested alone (nonsyndromic) or observed as a sign of other diseases (syndromic) such as craniofacial dysmorphism or CHARGE syndrome (coloboma, heart disease, atresia choanae, retarded growth and retarded development and/or central nervous system anomalies, genital hypoplasia, and ear anomalies and/or deafness).\(^4\) Ocular coloboma can be sporadic or transmitted as an autosomal recessive, autosomal dominant, or X-linked trait. Mutations in the following 7 genes have been identified in patients with coloboma: PAX6 (OMIM 607108),\(^3,6\) CHX10 (OMIM 142993),\(^7,8\) MAF (OMIM 177075),\(^9\) SHH (OMIM 600725),\(^10\) CHD7 (OMIM 608892),\(^11\) OTX2 (OMIM 600307),\(^12\) and GDF6 (OMIM 601147).\(^13\) Mutations in these genes, however, seem to account for only a fraction of colobomas.

SOX2 (OMIM 184429), a highly conserved gene located in 3q26.3-27.1, has a critical role in the development of the eye and brain.\(^14\) This single-exon gene encodes a protein with 317 residues, which consists of an N-terminal domain, a DNA-binding high-mobility group domain, and a transcriptional activation domain in the C-terminal. SOX2 protein, acting as a transcription factor, is expressed most notably in all stages of eye development.\(^15\) Precise regulation of SOX2 dosage is important in eye development in the mouse.\(^16\) SOX2 does not participate directly in closure of the optic cup fissure.\(^14\) Mutations in SOX2 are associated with microphthalmia or anophthalmia, esophageal atresia, and urogenital anomalies.\(^17\) In this study, a novel mutation in SOX2 was identified in a Chi-
nese family in which 2 members having the mutation exhibited clinical features.

**METHODS**

**PATIENTS AND CLINICAL DATA**

This Chinese family with 2 individuals who exhibited ocular coloboma lives in southern China. Informed consent in accord with the Declaration of Helsinki was obtained from the participating individuals before the study. This study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center, and systemic examinations were performed, and photographs were taken. Electroretinographic responses were recorded in available family members, consistent with the standards of the International Society for Clinical Electrophysiology of Vision.18

**MUTATION ANALYSIS**

Genomic DNA was prepared from peripheral leukocytes. Three pairs of primers were used to amplify the coding exon and adjacent intron region of SOX2 (NCBI human genome build 36.2, NC_000003.10 for genomic DNA, NM_003106.2 for messenger RNA, and NP_003097.1 for protein). The primer sequences were as follows: SOX2-AF: 5'-CGCCCTCCCTCTCTC-3'; SOX2-AR: 5'-CGCCCTCCCTCTCTC-3'; SOX2-BF: 5'-GGGCGCCGAGTGGAAACTT-3'; and SOX2-BR: 5'-GGGTGCCCTGCTGCGAGTA-3'.

The nucleotide sequence of SOX2 was determined with a cycle sequencing kit (BigDye Terminator, version 3.1; Applied Biosystems, Foster City, California) according to the manufacturer’s recommendations, on a genetic analyzer (ABI 3100; Applied Biosystems). Sequencing results from patients and SOX2 consensus sequence (NC_000003.10) were imported into the SeqManII program of the Lasergene package (DNAstar Inc, Madison, Wisconsin) and aligned to identify variations. Variation was confirmed with bidirectional sequencing. Mutation description followed the nomenclature recommended by the Human Genetic Variation Society (http://www.hgvs.org/mutnomen/). The mutation detected was further evaluated in available family members and 103 unaffected control individuals by restriction fragment length polymorphism using an extra pair of primers: SOX2-DF: 5'-CCCCCGGCGCAGGATACAT-3' and SOX2-DR: 5'-TCGGCGCCGGGAGATACAT-3'. Polymerase chain reaction products (448 base pairs [bp]) harboring the heterozygous c.695C>A mutation were digested with restriction endonuclease BSP1286-I. Wild amplicons were digested into 297 and 151 bp while the mutant could not be cut as the mutation erases the enzyme recognition site. Therefore, digested amplicons from patients with heterozygous mutations had 3 electrophoretic bands (448, 297, and 151 bp), but those from unaffected control individuals had 2 bands (297 and 151 bp).

**Figure 1.** Mutation identified in SOX2. A, Heterozygous mutation in SOX2 was exclusively detected in affected Chinese family members. B, The mutation was also detected by polymerase chain reaction–restriction length fragment polymorphism. Lane 1, Sample from unaffected control individual (NC); lanes 2 through 4, samples from family members; lane 5, uncut amplicons. Arrow indicates proband. C, The mutation involved a highly conserved residue.
RESULTS

A novel heterozygous missense mutation, c.695C>A, was identified in SOX2 of the proband (II:1) and his father (I:2) (Figure 1A). The nucleotide substitution would result in replacement of threonine by asparagine at codon 232 (ie, p.Thr232Asn). This mutation was not identified in his mother (I:1) or in 103 unaffected control individuals at polymerase chain reaction–restriction fragment length polymorphism analysis (Figure 1B). The threonine at position 232 is highly conserved for SOX2, as demonstrated by analysis of 9 orthologs from different vertebrate species (Figure 1C), but it is not conserved in other members of the SOX family.

The proband was a 14-year-old boy. He had microcornea (horizontal diameter, 7 mm), iris hypoplasia, and pupil malformation of the right eye. The lens and fundus of the right eye were not visible owing to anomalies of the iris and pupil (Figure 2A). The horizontal diameter of the left cornea was 11 mm. A keyhole-like pupil owing to an inferior iris coloboma was noted in the left eye since early childhood (Figure 2B). A large choroidal coloboma was observed on the left fundus (Figure 2C). B-scanning demonstrated axial length of 22.19 mm OD and 20.72 mm OS, which is shorter than the general population mean (SD) for axial length measurements (23.35 [2.50] mm). In addition, B-scanning revealed a 10 x 8.29-mm cystic structure behind the eyeball (Figure 2D). Magnetic resonance imaging confirmed the colobomatous orbital cyst, with relatively normal structure of other eye tissues including chiasm, optic nerves, and extraocular muscles (Figure 3).

The boy was born at full term after a normal delivery. The parents were not consanguineous. He began to speak at 12 months of age, and started to walk at 14 months of age. No history of seizure was reported. At age 14 years, he was 153 cm tall and weighed 40 kg, within the normal range of the population mean (SD) for height and weight (155.6 [7.83] cm and 42.56 [6.73] kg, respectively). Systemic examination revealed penoscrotal hypospadias without micropenis or cryptorchidism. Serum levels of human growth hormone, thyroid-stimulating hormone, and follicle-stimulating hormone were within the normal range. Results of Madsen electronics testing showed normal hearing bilaterally. B-scanning demonstrated no hepatic or renal abnormalities. Magnetic resonance imaging revealed a 53 x 42-mm arachnoid cyst located in the middle cranial fossa on the left side, which compressed the left frontal lobe and temporal lobe (Figure 3C and D). Other brain tissues, including the pituitary gland, were comparatively normal (Figure 3).

The proband’s 42-year-old father had normal cornea (horizontal diameter, 11.5 mm), inferior iris coloboma, keyhole pupil, and punctate and lamellar lens opacities of the right eye (Figure 2E and F). Choriotretinal hypoplasia was noted in the inferior fundus, which implied a mild variant of choroidal coloboma (Figure 2G). Ultrasoundographic biomicroscopy demonstrated inferior absence of the iris and of lens zonular fibers (Figure 2H). At optical coherence tomography, a vertical scan through the optic disc revealed thinner inferior retina (66 µm) in the right eye compared with that (141 µm) in the left eye (Figure 2I). A B-scan recorded axial length of 26.63 mm OD and 25.04 mm OS. His left eye was normal without any recognizable malformation. Standard full-field electroretinography showed normal rod and cone responses in both eyes. Magnetic resonance imaging in the father did not reveal any malformation in the brain and orbit. No other phenotypes were noted at systemic examination. Ocular and systemic examinations in the proband’s mother yielded normal findings.

COMMENT

A novel mutation in SOX2, c.695C>A (p.Thr232Asn), was identified in 2 patients (father and son) from a Chinese family with typical ocular coloboma. The proband had microcornea with iris hypoplasia in the right eye, and band keratopathy (postoperative change), an inferior coloboma involving the iris and chorioretina, and a retrobulbar cystic mass in the left eye. His father had an inferior iris coloboma, congenital cataract, and inferior choriocapillaris atrophy in the right eye.
In 2003, mutations in SOX2 were first reported by Fantes et al\textsuperscript{21} to be responsible for anophthalmia.\textsuperscript{21} To date, 23 mutations in SOX2 have been described, including 7 nonsense, 4 missense, and 12 frameshift mutations, in patients with anophthalmia or microphthalmia.\textsuperscript{21-31} The 4 missense mutations at the N-terminal or high-mobility group domain occurred at an evolutionarily highly conserved position that would affect DNA binding activity.\textsuperscript{24,27-29} To our knowledge, the p.Thr232Asn mutation identified in this study is the first missense mutation located in the transcriptional activation domain, which is functionally essential in order for SOX2 to activate target genes.\textsuperscript{32} The p.Thr232Asn mutation replaced a hydrophobic residue with a hydrophilic residue, resulting in a change at the protein level with a residue weight of zero.\textsuperscript{33} In theory, the p.Thr232Asn mutation would be expected to influence the function of SOX2 protein if it is expressed.

To date, mutations in SOX2 have been identified in 37 patients, manifested with at least 1 of the following ocular anomalies: cataract, achiasma, microphthalmia, anophthalmia, sclerocornea, dysplastic optic disc, or persistent pupillary membrane.\textsuperscript{21-31} Extraocular findings may involve seizures, renal duplex, cranial anomalies, esophageal atresia, micropenis and cryptorchidism, penoscrotal hypospadias, sensorineural hearing loss, delayed speech development, learning difficulty, delayed motor mile-

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\caption{Magnetic resonance images of the orbit and brain in the proband. A, Coronal image demonstrates a retrobulbar colobomatous cyst on the right (arrow). B, Sagittal oblique image reveals that the right orbital cystic mass had a clear boundary (arrow) and that the pituitary gland was normal. C and D, Axial T1-weighted and T2-weighted images, respectively, disclosed a left-sided arachnoid cyst (arrows).}
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stones, suprasellar cyst, pineal cyst, small pituitary gland, hippocampal malformation, hypothalamic hamartoma, and deficiencies of growth hormone, thyrotropin, and follicle-stimulating hormone.\textsuperscript{21-31} Previously, mutations in PAX6, CHX10, MAF, SHH, CHD7, OTX2, and GDF6 have been reported in patients with ocular coloboma. Arachnoid cyst can occur independently or in association with several diseases.\textsuperscript{34} but only mutation in SPG4 (OMIM 604277) is reported to associate with arachnoid cyst.\textsuperscript{35}

To our knowledge, SOX2 mutation has not been identified in 2 generations with ocular colobomas, and typical ocular coloboma and arachnoid cyst have not been reported in patients with SOX2 mutation.

Mutations in SOX2 previously have been associated with male genital tract abnormalities, and thus far, all mutations have been detected in sporadic cases or siblings. It would be expected that the SOX2 mutation might affect fertility. In this study, the father had ocular coloboma but without genital anomaly, and he passed the mutation to his son. This may provide useful information for study of the SOX2 mutation as it relates to genitaluinary development.

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