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## Novel *SOX2* Mutations and Genotype-Phenotype Correlation in Anophthalmia and Microphthalmia

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### Abstract

*SOX2* represents a High Mobility Group domain containing transcription factor that is essential for normal development in vertebrates. Mutations in *SOX2* are known to result in a spectrum of severe ocular phenotypes in humans, also typically associated with other systemic defects. Ocular phenotypes include anophthalmia/microphthalmia (A/M), optic nerve hypoplasia, ocular coloboma and other eye anomalies. We screened 51 unrelated individuals with A/M and identified *SOX2* mutations in the coding region of the gene in 10 individuals. Seven of the identified mutations are novel alterations, while the remaining three individuals carry the previously reported recurrent 20-nucleotide deletion in *SOX2*, c.70del20. Among the *SOX2*-positive cases, seven patients had bilateral A/M and mutations resulting in premature termination of the normal protein sequence (7/38; 18% of all bilateral cases), one patient had bilateral A/M associated with a single amino acid insertion (1/38; 3% of bilateral cases), and the final two patients demonstrated unilateral A/M associated with missense mutations (2/13; 15% of all unilateral cases). These findings and review of previously reported cases suggest a potential genotype/phenotype correlation for *SOX2* mutations with missense changes generally leading to less severe ocular defects. In addition, we report a new familial case of affected siblings with maternal mosaicism for the identified *SOX2* mutation, which further underscores the importance of parental testing to provide accurate genetic counseling to families.

### Keywords

Anophthalmia; microphthalmia; *SOX2*.

### INTRODUCTION

Anophthalmia, defined as the absence of a globe in the orbit, and microphthalmia, defined as reduced size of the globe, are malformations of the eye seen in approximately 1–3/10,000 births. Anophthalmia and microphthalmia (A/M) are considered to represent a single continuum; the severity of microphthalmia varies significantly among affected individuals and clinical anophthalmia is believed to be the manifestation of extreme microphthalmia with ocular remnants visible only on imaging of the orbit. Chromosomal anomalies account

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for 23–30% of patients with A/M. Excluding those with chromosomal anomalies, 73–95% of individuals with A/M have other associated anomalies; only 20–45% of these can be attributed to a known syndrome [Clementi et al., 1996; Källén et al., 1996; Forrester and Merz, 2006].

Mutations in multiple genes have been implicated in A/M including *SOX2*, *PAX6*, *SIX6*, *CHD7*, *CHX10*, *BCOR*, *RAX*, *PITX3*, and *OTX2* [Hever et al., 2006; Verma and Fitzpatrick, 2007]. Mutations in *SOX2* appear to represent the most common cause of A/M. Previous studies have reported coding region mutations in 10–15% of individuals with bilateral severe eye malformations, including anophthalmia, microphthalmia, and coloboma [Fantes et al., 2003; Ragge et al., 2005]; one study also identified whole gene deletions in ~10% of patients who had severe A/M without coding region mutations in *SOX2* [Bakrania et al., 2007].

Most of the *SOX2* coding region mutations are represented by nucleotide substitutions or deletions/insertions resulting in a premature truncation of the normal protein. The majority of these truncating mutations are observed *de novo* in patients. Several missense mutations in *SOX2* have also been identified and are predicted to affect the DNA-binding or transactivation domains of this protein, which are critical to its activity [Ragge et al., 2005; Faivre et al., 2006; Kelberman et al., 2006; Williamson et al., 2006; Sato et al., 2007; Wang et al., 2008]. Three familial cases of A/M with a *SOX2* mutation (two truncating, one missense) identified in the proband and unaffected mother of affected siblings or half-siblings have been reported; maternal mosaicism was proposed for all three cases [Faivre et al., 2006; Chassaing et al., 2007; Schneider et al., 2008]. In addition, there have been three cases of milder eye defects associated with *SOX2* missense mutations inherited from a father; two of the fathers were unaffected and the third had unilateral eye defects similar to those seen in his son [Kelberman et al., 2006; Wang et al., 2008].

The spectrum of phenotypes associated with *SOX2* mutations ranges from severe forms of anophthalmia syndrome characterized by bilateral anophthalmia with mild dysmorphic facial features, genital anomalies in males, brain malformations, learning disabilities, and motor delay, to normal ocular phenotypes which may be observed in individuals with the same mutations [Zenteno et al., 2006; Chassaing et al., 2007]. Additional ocular features observed in *SOX2*-positive patients include cataract, coloboma, optic nerve hypoplasia, various forms of anterior segment dysgenesis, retinal and chorioretinal dystrophy, and high myopia [Kelberman et al., 2006; Bakrania et al., 2007]. The range of observed phenotypes is consistent with the critical role that *Sox2* was shown to play in vertebrate embryonic development. Studies in animal models demonstrated that *Sox2* deficiency leads to abnormal neurogenesis, defects in inner ear development, loss of pluripotency of embryonic stem cells [Ferri et al., 2004; Kiernan et al., 2005; Kelberman et al., 2006; Masui et al., 2007] and, most notably, a range of ocular phenotypes depending on the dosage of *Sox2* in mice [Taranova et al., 2006].

In this report we present ten additional cases of mutations in *SOX2* discovered in patients with anophthalmia/microphthalmia and a review of all *SOX2* mutations reported to date. Our analysis suggests that missense mutations in *SOX2* appear to result in milder phenotypes, similar to the dosage-dependent ocular phenotypes observed in mice. In addition, we report two new cases of familial anophthalmia associated with *SOX2* mutations and parental mosaicism in one case.

## MATERIALS AND METHODS

This human study was approved by the Institutional Review Boards of Children's Hospital of Wisconsin and Albert Einstein Healthcare Network. Cases were identified and clinical data collected through the A/M Clinical Registry at Albert Einstein Medical Center, Philadelphia, or as part of the Genetic Studies of Human Developmental Disorders at the Medical College of Wisconsin, Milwaukee. Clinical data was assembled by review of medical records and/or clinical evaluation by one of the authors (AS).

DNA samples were extracted from blood or buccal samples obtained from 51 individuals with A/M. Of the 51 cases screened, 38 had bilateral A/M and 13 had unilateral A/M. The *SOX2* coding region was amplified to produce two overlapping PCR products using the following primers: set 1 forward, 5'-AGTCCCGGCCGGCCGAG-3', and set 1 reverse, 5'-GGTAGCCCAGCTGGTCCTG-3', and set 2 forward, 5'-CAAGACGCTCATGAAGAAGG-3', and set 2 reverse, 5'-TACTCTCCTCTTTTGCACCC-3'. The PCR products were sequenced in both forward and reverse directions using Big Dye Terminator v3.1 with Applied Biosystems 3730 DNA sequencer. Chromatograms were examined manually and using Mutation Surveyor software (SoftGenetics). All initially identified changes were confirmed by additional independent PCR and sequencing experiments.

To obtain control data for normal variation in *SOX2* in different ethnic populations, we obtained sequence data from 90 Caucasian, 87 African American and 90 Hispanic control individuals using the above approach. The control DNA samples were obtained from the Coriell Institute for Medical Research (Camden, NJ).

## RESULTS

Sequencing of *SOX2* in a cohort of 51 unrelated individuals affected with bilateral (38) or unilateral (13) anophthalmia/microphthalmia (Figure 1) revealed *SOX2* mutations in ten patients (19.6%). These mutations were not seen in the ninety Caucasian, eighty-seven African American and ninety Hispanic control DNA samples. The phenotypic and genetic details of the affected families and identified mutations are presented below and in Table I and Figures 1 & 2.

Patient 1 is a 2-year-old Caucasian female with right anophthalmia and left microphthalmia, coloboma, glaucoma, and cataract with some vision in the left eye. Clinical descriptions were obtained through available medical records. Growth parameters were not available for review. The patient had adhesions of the vagina treated with progesterone cream. She is noted to have small ears. Brain MRI, cardiac exam and renal ultrasound were normal. Speech was delayed with good comprehension and walking was late with a "drunken" gait. This patient was found to have a c.16G>T mutation (please see chromatogram in Figure 2A and mutation position in Figure 2B) which creates a stop codon and leads to truncation of the protein product at 5 amino acids. Parents have not been tested.

Patient 2 is a 2-year-3 month-old Hispanic female with bilateral severe microphthalmia. At 2 years 3 months of age, her weight was 13 kg (50<sup>th</sup>-75<sup>th</sup> centile), height was 84.5 cm (5<sup>th</sup>-10<sup>th</sup> centile) and head circumference 46.5 cm (10<sup>th</sup>-25<sup>th</sup> centile). Renal ultrasound was normal. MRI of the head revealed a hamartoma of the tuber cinereum. Motor development is delayed and she has a wide based stance; she crawled at 12 months and is not yet walking. She babbles, has better receptive than expressive language, and is friendly and interactive. Family history is remarkable for a 14 year old maternal half-sister with mental retardation and unilateral anophthalmia. There is no known consanguinity. The patient was found to have the previously reported c.70del20 mutation (Figure 2), a 20-nucleotide deletion, which

results in frameshift and truncation of the protein product at 23 amino acids. The mother was found to be mosaic for the c.70del20 mutation in three independent sequencing reactions and clinical testing confirmed that the affected sibling also carries this mutation.

Patient 3 is a 9-year-old Hispanic male with bilateral clinical anophthalmia and no vision. He was first evaluated at 10 months of age. At that time height and head circumference were below the 3<sup>rd</sup> centile (67.5 cm and 42 cm, respectively). Dysmorphic features including microcephaly, slightly cupped ears, mild dolicocephaly, lateral flaring of the eyebrows, and widely-spaced central incisors were noted. He has a single café-au-lait mark. He has micropenis and cryptorchidism. Cardiology evaluation is pending following the new observation of a grade 2/6 ejection murmur at the left sternal border. Head CT was normal. He has significant motor and cognitive delay and he has been diagnosed with autism spectrum disorder. Based on clinical examination, a possible diagnosis of Lenz syndrome was discussed with the family prior to *SOX2* studies. This patient was also found to be heterozygous for the previously reported c.70del20 mutation (see above and Figure 2B). The patient's mother was tested and does not carry this mutation. The patient's father is unavailable for testing.

Patient 4 is an 8-year-old Caucasian male with bilateral clinical anophthalmia who was evaluated at 2 years 9 months of age. His weight was 25<sup>th</sup>–50<sup>th</sup> centile (13.7 kg), height was 25<sup>th</sup> centile (91.5 cm) and head circumference was 10<sup>th</sup>–25<sup>th</sup> centile (47.5 cm). Clinical examination revealed hypotonia, foreskin adhesions, bilateral cutaneous 2–3 toe syndactyly and mild pectus excavatum. Brain MRI was normal. He has significant global developmental delay. He was sitting and crawling at around 3 years of age and is still unable to walk. He has very limited speech and has been diagnosed with verbal apraxia. He has a history of febrile seizures which have resolved. This patient also carries the previously reported c.70del20 mutation (see above and Figure 2B). Both parents were tested clinically and are negative for the mutation seen in their son.

Patient 5 is an almost 7-year-old Caucasian male with bilateral anophthalmia. Clinical descriptions were obtained through available medical records. His height is below the 3<sup>rd</sup> centile (104.9 cm) with weight at the 3<sup>rd</sup> to 5<sup>th</sup> centile (17.9 kg) and he demonstrated low growth hormone levels (IGF-1 and IGF-binding protein 3). The patient has a history of micropenis at birth treated with testosterone. He has reflux and has been fed via gastrojejunum tube since age 15 months. In addition, he has had recurrent episodes of apnea. Muscle biopsy at 20 months of age showed mitochondrial accumulation with secondary glycogen and lipid accumulation. Brain MRI showed a cavum septum pellucidum, non-specific periventricular white matter signal abnormality, and abnormal configuration of the pituitary and sellar structures. He has significant global developmental delay; he rolls, but is unable to sit, crawl, or walk, and has limited speech. Muscle biopsy showed mitochondrial accumulation. This patient was found to have a c.487ins2 mutation (Figure 2) which leads to frameshift and truncation of the protein product at 163 amino acids. Both parents were tested and did not carry the mutation seen in their son.

Patient 6 is a 4-year-old Caucasian male with left anophthalmia and right microphthalmia (Figure 1A). Clinical descriptions were obtained through available medical records. Height and weight are in the normal range (97.5 cm at the 10<sup>th</sup>–25<sup>th</sup> centile and 16.6 kg at the 50<sup>th</sup>–75<sup>th</sup> centile, respectively). The patient has a history of micropenis at birth, treated with testosterone, and left undescended testicle repaired surgically. He has a history of pyloric stenosis and was fed via gastrostomy tube from age 2 months to 1 year due to poor oral intake. Renal ultrasound was normal. Brain MRI showed a 9 mm suprasellar mass posterior to the pituitary stalk (likely a benign teratoma), prominent cerebrospinal fluid spaces in the posterior fossa, and a cavum septum pellucidum. A tonic-clonic seizure disorder was

diagnosed at age 3 years. His gross motor skills are significantly delayed; he first walked at 3½ years of age and currently takes only 8–10 steps independently. He also has mild delays in speech and fine motor milestones. This patient was found to have a c.540C>G mutation (Figure 2) which creates a stop codon and results in truncation of the protein product at 180 amino acids. Both parents were tested and were negative for the mutation seen in their son.

Patient 7 is an 8-year-10 month-old Caucasian male with bilateral anophthalmia. Clinical examination revealed normal growth: head circumference at the 10<sup>th</sup>–25<sup>th</sup> centile (51 cm), height at 5<sup>th</sup>–10<sup>th</sup> centile (122.4cm) and weight at 10<sup>th</sup>–25<sup>th</sup> centile (26.6kg). Dysmorphic features including short, narrow palpebral fissures, slightly cupped ears, maxillary overbite with crowded teeth and prominent central incisors, retrognathia, long chest, 5<sup>th</sup> finger clinodactyly and hyperextensible joints were noted. He has a deep sacral dimple with normal ultrasound of the lumbosacral spine. A spigelian hernia was noted. Echocardiogram shortly after birth revealed a patent ductus arteriosus, mild tricuspid regurgitation, atrial septum bowing into right atrium and moderately severe pulmonary hypertension. Cranial MRI was normal. He has a history of complex partial seizures. He has gross motor delay with balance difficulties, but can walk independently. His speech was significantly delayed and he continues to have an expressive communication disorder. This patient was found to be heterozygous for the c.937del1 (Figure 2), a 1-nucleotide deletion which causes frameshift and leads to truncation of the protein product at 313 amino acids followed by 57 erroneous residues due to frameshift. Parental samples were not available for testing.

Patient 8 is a 9-year-old Hispanic male with bilateral anophthalmia. His height is at the 3<sup>rd</sup> centile (11.8cm), weight is at the 3<sup>rd</sup> centile (20.5kg) and head circumference is at the 3<sup>rd</sup>–5<sup>th</sup> centile (50.4 cm), despite treatment with growth hormone. He demonstrated low growth hormone levels (IGF-1 and IGF-binding protein 3) at 1 year 6 months of age and has been treated since. Dysmorphic features including coarse facial features with deep set, widely spaced eyes, full eye brows, thick eye lashes, fleshy ear lobes, and a large mouth with widely spaced teeth. Acanthosis nigricans was noted. He has normal hearing but little conversational speech. MRI of the brain showed excess extra-axial fluid and an ectopic neurohypophysis with an underdeveloped pituitary gland. At approximately 7 years of age he was diagnosed with panhypopituitarism and began treatment with thyroxin in addition to growth hormone. He has significant insomnia, global developmental delay and autistic features. He walked at age 5½ years; currently gait is described as “shuffling” and he needs support when walking outside. The mother had a subsequent pregnancy, a half-sibling to the patient, with a prenatal diagnosis of a male fetus with right anophthalmia which was terminated. Autopsy revealed left retinal dysplasia and right microphthalmia with absence of the optic nerve. The patient was found to be heterozygous for a c.69ins3 mutation (Figure 2), an in-frame insertion adding a single glycine at position 23. Clinical testing of autopsy tissue performed on the affected half-sibling did not identify the mutation, but allele drop-out could not be ruled-out. Testing of a blood sample from the mother did not show any signs of mosaicism. The father is unavailable for testing.

Patient 9 is a 3-year-6-month-old Caucasian male with unilateral (left) clinical anophthalmia (Figure 1B). His current height and weight are in the normal range (96 cm at the 25<sup>th</sup> centile and 13.6 kg at the 10<sup>th</sup> centile, respectively), but his head circumference is less than the 3<sup>rd</sup> centile (47.4 cm). On clinical examination he was noted to have microcephaly, mild 2,3 toe syndactyly and sutural ridging. He has micropenis and a normal renal ultrasound. Brain MRI revealed that the lateral ventricles are mildly prominent with no evidence of hydrocephalus. Initial newborn hearing screening was normal but he has failed subsequent hearing screens. Speech and motor development are mildly delayed and he has an unusual gait. He walked at 20 months of age but continues to exhibit an unsteady gait. His receptive language skills have tested in the normal range but he exhibits about a 25% delay in expressive speech.

Endocrinology evaluation was performed with no pituitary deficiency identified. The patient was found to be heterozygous for a c.131C>G mutation (Figure 2) that results in the substitution of arginine for proline at position 44. Both parents were tested and were negative for the mutation seen in their son.

Patient 10 is a 3-year-old Haitian (Black) female with unilateral (left) microphthalmia. She is adopted from Haiti and no medical history is available until she was taken to an orphanage at age 2 years. Her growth parameters are in the normal range with height of 90 cm (10<sup>th</sup>–25<sup>th</sup> centile), weight of 12.6 kg (10<sup>th</sup>–25<sup>th</sup> centile) and head circumference of 49.2 cm (25<sup>th</sup>–50<sup>th</sup> centile). Dysmorphic features include facial asymmetry and marked micrognathia with difficulty opening her mouth fully. She has had surgery to break and expand her jaw with follow-up stretching exercises. Teeth are unremarkable. She has a leg-length discrepancy of 3 cm on the left with an area of scar tissue on the thigh, thought to be due to a bone infection while in the orphanage. Renal ultrasound and cervical, thoracic and lumbar spine X-rays were normal; head imaging studies were not performed. Development is typical. She has no motor delays or ataxia and speaks in full sentences with no difficulties. This patient was found to have a heterozygous mutation, c.859G>C (Figure 2), a missense mutation which results in the substitution of proline for alanine at position 287. Parental samples were not available for testing.

## DISCUSSION

Screening of *SOX2* in fifty-one unrelated patients with bilateral or unilateral anophthalmia/microphthalmia identified mutations in ten individuals, 21% of bilateral A/M and 15% of unilateral A/M. Out of these ten mutations, seven represent new alterations in *SOX2* with the c.16G>T substitution in Patient 1 being the most 5' *SOX2* mutation reported to date. Interestingly, despite a dramatic difference in the predicted effects on the amino acid composition of mutant proteins between Patient 1 (1.6% of the normal *SOX2* protein) and Patient 7 (99% of the normal *SOX2* protein), the phenotypes of both patients appear to be similar and severe, with bilateral anophthalmia/microphthalmia seen in both cases. The phenotypes of the other five patients with nonsense/frameshift mutations (Patients 2–6) also appear to be consistent with the previously reported cases with mutations resulting in truncation of the *SOX2* protein (Table II). Of special interest is the 20-nucleotide deletion, c.70del20, which was identified in three unrelated families with severe microphthalmia/anophthalmia syndrome. This deletion has been previously reported and we present further evidence of its high recurrence. This deletion accounts for 30% of *SOX2* mutations in our population and 18% of all *SOX2* cases reported to date; this mutation alone explains 8% of bilateral anophthalmia/microphthalmia cases in this study and, therefore, development of additional diagnostic tests for an accurate detection of this deletion may be warranted.

The other identified changes include a one amino acid-insertion and two novel missense mutations. The one amino acid insertion involving glycine at position 23 in Patient 8, c.69ins3, may represent a new type of *SOX2* mutation with a potentially unique mechanism. The normal human protein has 5 glycine residues in its N-terminal region; one additional residue is inserted in the mutant protein. This mutation was not found in any of the control samples of diverse ethnic origins including ninety individuals of Hispanic descent, matching this patient's ethnicity. At the same time, this region does not appear to be conserved in different species as the mouse *SOX2* protein contains seven and the *Xenopus* protein has two glycines in the same area. The proband was reported to have an affected half-sibling, but clinical genetic testing of the available autopsy tissue proved to be difficult and failed to produce a definitive answer in regards to presence/absence of this mutation in the half-sibling sample. Examination of a blood sample from the mother did not show any signs of mosaicism while the father is not available for testing. The c.69ins3 insertion may represent

a causative or contributing mutation in this family or it could be a very rare polymorphism. A contributing mutation is not sufficient to cause a phenotype alone, but together with another mutation in a different gene, results in the observed disease. This type of digenic inheritance involving a gene which is typically implicated in autosomal dominant disease has been shown for the L185P mutation in *RDS*, which results in retinitis pigmentosa when combined with a heterozygous mutation in *ROM1* (Kajiwara et al., 1994; Dryja et al. 1997). Functional studies of this mutation as well as additional screenings of the *SOX2* gene in patients with A/M are likely to provide further insight into effects of this mutation.

Based upon their location, the missense changes in Patient 9 (P44R) and Patient 10 (A287P) are predicted to affect the high mobility group domain that mediates *SOX2* binding to DNA and the C-terminal region involved in transactivation and interactions with co-activators and other proteins, respectively [Kamachi et al., 2000; Nowling et al., 2000]. Therefore, these changes are predicted to disrupt the regulatory activities of this transcription factor. Both the arginine at position 44 and the alanine at position 287 were found to be conserved in different vertebrate species, which further supports their importance for the normal functioning of this protein.

Six cases of inherited *SOX2* mutations, three involving anophthalmia/microphthalmia, have been reported in the literature, with maternal mosaicism proposed for the families with A/M [Faivre et al., 2006; Kelberman et al., 2006; Chassaing et al., 2007; Schneider et al., 2008; Wang et al., 2008]. We report one additional case involving familial anophthalmia/microphthalmia associated with parental mosaicism (Family 2). This suggests a relatively high rate of familial *SOX2* mutations and underscores the importance of careful analysis of parental samples whenever a *SOX2* mutation is identified in a child.

Interestingly, missense mutations in our sample corresponded to less severe ocular phenotypes than nonsense or frameshift mutations. Both missense mutations were associated with unilateral anophthalmia and normal development of the contralateral eye. Analysis of the literature revealed that the association observed in our sample appears to be even more evident when all previously reported *SOX2* mutations are taken into consideration (Table II). Out of forty reported nonsense or frameshift mutations, 89% of eyes were anophthalmic or microphthalmic, 5% demonstrated some other, milder, ocular phenotype, and 6% of eyes were normal; similar frequencies were observed for partial/complete gene deletions, 90%, 5% and 5%, correspondingly. In contrast, patients carrying missense mutations were equally likely to display A/M (33% of eyes), other phenotypes (33%) or normal ocular development (33%). In addition, patients with missense mutations demonstrated fewer other developmental or systemic abnormalities. In our sample, Patient 10 showed no developmental issues and Patient 9 exhibited only mild delays in motor and speech development, as opposed to the significant speech and motor delays typically described in *SOX2* anophthalmia syndrome. Again, this correlation held true when analyzing previously reported *SOX2* mutations with global delay noted in only one out of eight patients. Considering all patients with *SOX2* mutations, patients with missense mutations averaged one additional major systemic feature, while patients with deletions, nonsense, or frameshift mutations averaged two additional major systemic features.

The phenotypic variability observed for *SOX2* mutations may be explained by the critical and broad role of this factor in ocular development as well as certain unique features of this protein. The *SOX* proteins contain the highly conserved High Mobility Group (HMG) domain, which interacts with DNA, and a C-terminal transactivation domain, which cooperates with other proteins to activate expression of downstream genes. As many other HMG-containing proteins, *SOX2* was shown to have low DNA-binding affinity and therefore requires additional proteins (co-DNA-binding factors) to facilitate this interaction

and achieve efficient and specific binding to target DNA sites [Scaffidi and Bianchi, 2001]. It is plausible to assume that due to this required interaction with multiple additional factors to execute both its DNA-binding and transactivation functions, performance of the mutant SOX2 proteins may be greatly modified based upon these additional interactions. Missense mutations in *SOX2* may affect certain interactions/functions of this protein to a greater extent than others and, therefore, may result in variable phenotypes based on the specific requirement/role of these interactions in eye development. Some of the identified members of the SOX2 pathway are also associated with A/M. The SOX2 and OTX2 proteins were recently shown to co-regulate expression of the *RAX* gene, thus connecting three known A/M genes in the same pathway [Danno et al., 2008].

Another possible explanation for the milder phenotypes seen in some patients is that missense mutations result in only partial loss-of-function and the less severe phenotypes may be due to a variable requirement of *SOX2* for different developmental processes. A similar phenomenon was observed in animal models: *Sox2*-deficiency in mice has been shown to be associated with variable phenotypes (Kiernan et al., 2005; Kelberman et al., 2006; Taranova et al., 2006). Most notably, Taranova et al. (2006) generated a *Sox2* allelic series in the mouse and revealed dosage-dependent variation in ocular phenotypes. Variable expressivity of *Sox2* deficiency in mice in relation to the ocular phenotype was also demonstrated by Kelberman and coauthors (2006) who reported anterior pituitary defects and male impaired fertility but normal eye development in mutant mice heterozygous for a *Sox2* deletion.

Functional analyses of human *SOX2* mutations may provide new insights into potential disease mechanisms as well as the normal function of this important transcription factor. Also, further studies involving examination of *SOX2* in a variety of ocular phenotypes may identify additional mutations and help to define the role of this gene in human eye disease.

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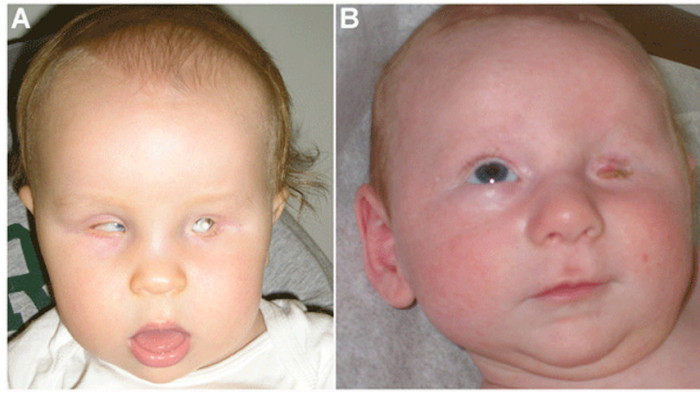
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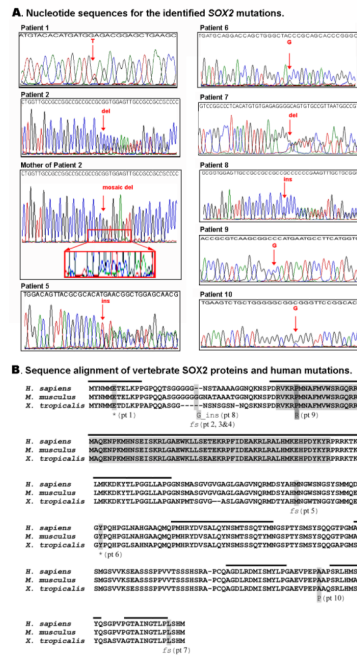
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**Figure 1. Patient photographs**

**A)** Patient 6 at 14 months of age with left anophthalmia (orbital expander in place) and right microphthalmia. **B)** Patient 9 at 2 months of age with left clinical anophthalmia and normal right eye.



**Figure 2.**

**A) Nucleotide sequences for the identified SOX2 mutations.** Fragments of chromatograms obtained for different regions of the *SOX2* gene using patient and parent (mosaic mother, family 2) DNA are shown. Mutation positions are indicated with red arrows. Mosaicism is demonstrated in the mother of Patient 2 by the small peaks corresponding to the frameshift at position 70 under the large peaks corresponding to the normal sequence, as opposed to the relatively equal peaks seen in Patient 2 with heterozygote status. The region corresponding to the start of the maternal mosaic sequence is enlarged for easier viewing. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).] **B) Sequence alignment of vertebrate SOX2 proteins and human mutations.** Human, mouse and frog sequences are shown; positions and details of identified human mutations are indicated at the bottom of the alignment; High Mobility Group (HMG) DNA-binding domain (comprising seventy-two amino acids) is shown in grey; regions of high homology shared by all Group B SOX proteins, SOX1-3, are marked with a black line at the top of the alignment. fs- frameshift, \*stop codon, ins- insertion, pt- patient.

Table 1

Summary of clinical features and *SOX2* genotype for mutation-positive patients from this study.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
<b>Sex</b>	Female	Female	Male	Male	Male	Male	Male	Male	Male	Female
<b>Race/ethnicity</b>	Caucasian	Hispanic	Hispanic	Caucasian	Caucasian	Caucasian	Caucasian	Hispanic	Caucasian	Haitian (Black)
<b>Age at eval</b>	2	2	9	2	7	4	8	9	3	3
<b>Ocular defects</b>										
<b>Right</b>	Anophthalmia	Severe Microphthalmia	Anophthalmia	Anophthalmia	Anophthalmia	Microphthalmia	Anophthalmia	Anophthalmia	Normal	Normal
<b>Left</b>	Microphthalmia, coloboma, glaucoma, cataract	Severe Microphthalmia	Anophthalmia	Anophthalmia	Anophthalmia	Anophthalmia	Anophthalmia	Anophthalmia	Anophthalmia	Severe Microphthalmia
<b>Height</b>	Unknown	5th–10th	<3rd	25th	<3rd	10th–25th	5th–10th	3rd	25th	10th–25th
<b>Motor Delay</b>	Y	Y	Y	Y	Y	Y	Y	Y	Mild	N
<b>Developmental Delay</b>	Mild	Y	Autism Spectrum Disorder	Y	Y	Mild	Y	Autism Spectrum Disorder	Mild	N
<b>Neuroimaging Results</b>	Normal	Hamartoma of tuber cinereum	Normal	Normal	Cavum septum pellucidum, nonspecific periventricular white matter signal abnormality	Prominence of CSF spaces in posterior fossa; cavum septum pellucidum	Normal	Excess extra-axial fluid	Prominent lateral ventricles	Not available
<b>Pituitary Anomalies</b>	N	N	N	N	Abnormal configuration of pituitary and sellar structures	9mm suprasellar mass posterior to pituitary stalk(likely benign teratoma)	N	Ectopic neurohypophysis underdeveloped pituitary, pan-hypopituitarism	N	N
<b>Genital Anomalies</b>	Vaginal adhesions	N	Micropenis, cryptorchidism	Foreskin adhesion	Micropenis	Micropenis, L. cryptorchidism	N	N	Micropenis	N
<b>Other</b>	Small ears	Half-sister with unilateral anophthalmia and MR	Heart murmur	2,3 toe syndactyly, febrile seizures, mild pectus excavatum	Apnea, reflux, low growth hormone, mito accumulation on muscle bx	Seizure disorder, pyloric stenosis, feeding disorder	Cardiovascular defect, seizures, hernia	Acanthosis nigricans, TAb of half-brother with anophthalmia	Microcephaly, 2,3 toe syndactyly, hearing loss	Severe micrognathia
<b>SOX2 mutation</b>	c.16G>T	c.70del20	c.70del20	c.70del20	c.487ins2	c.540C>G	c.937del1	c.69ins3	c.131C>G	c.859G>C
nucleotide										

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
protein/effect	stop codon/truncation at 5 aa	frameshift/truncation at 23 aa	frameshift/truncation at 23 aa	frameshift/truncation at 23 aa	frameshift/truncation at 163 aa	stop codon/truncation at 180 aa	frameshift/truncation at 313 aa	1 aa insertion, in frame/G23insG	missense/P44R	missense/A287P

Table II

Genotype and phenotype information for all patients with *SOX2* mutations reported to date.

Reference	Mutation	R Eye	L Eye	Delay	Other	Family History
<b>NONSENSE/FRAMESHIFT/TRUNCATION MUTATIONS</b>						
<b>this study patient 1</b>	c.16G>T	AN	MI, CA, COL, GL	MD	GU	
Bakrania 2007	c.53C>A	AN	AN	MD	EN, GI, MA	
Pedace 2009	c.59_60insGG	AN	MI, COL	MD	GU	
Kelberman 2006	c.60insG	AN	AN	GD	BA, EA, MA	Parents DNA normal
Ragge 2005	c.67del23	AN	AN	GD	BA, SS, SZ	Parents DNA normal
Chassaing 2007	c.70del17	AN	AN		EA	Mutation seen in affected sibling, unaffected mother (mosaicism proposed)
Chassaing 2007	c.70del17	NOR	NOR	NOR	BA, VA	Sibling of above
Zenteno 2005	c.70del20	AN	AN		BA	
Bakrania 2007	c.70del20	AN	ASD, CA, COL, GL	GD	EA, GU, TEF	
Bakrania 2007	c.70del20	AN	AN	NOR		
Kelberman 2006	c.70del20	MI	AN	GD	BA, EA	Parents DNA normal
Zenteno 2006	c.70del20	NOR	AN		TEF, GU	Monozygotic twin below: Parents DNA normal
Zenteno 2006	c.70del20	NOR	NOR		TEF	Monozygotic twin of above
Kelberman 2008	c.70del20	AN	AN	NOR	EN	Parents DNA normal
<b>this study patient 2</b>	c.70del20	MI	MI	MD	BA	Half-sister is affected; unaffected mother carries mutation (mosaic)
<b>this study patient 3</b>	c.70del20	AN	AN	GD	GU, SS	Mother's DNA normal
<b>this study patient 4</b>	c.70del20	AN	AN	GD	GU, SZ	Parents DNA normal
Williamson 2006	c.163C>T	AN	AN		EA, GU, TEF	
Kelberman 2008	c.181C>T	AN	AN	GD	EN	Parents DNA normal
Bakrania 2007	c.188delA	CA, COL, CR, ET, MC, MY, PU	MY, ONH, PU	NOR	BA	
Fantes 2003; Ragge 2005	c.248C>A	AN	MI, CA, PU	GD	BA, MA, SZ	Parents DNA normal
Fantes 2003; Ragge 2005	c.277G>T	AN	MI, SC	MD	MA	Parents DNA normal
Bakrania 2007	c.285dupG	AN	AN			
Zhou 2008	c.310G>T	AN	AN	GD	BA, SS	

Reference	Mutation	R Eye	L Eye	Delay	Other	Family History
<b>NONSENSE/FRAMESHIFT/TRUNCATION MUTATIONS</b>						
Kelberman 2006	c.387delC	COL	MI	GD	BA, EA, GU, SD	Parents DNA normal
Hagstrom 2005	c.463C>T	AN	AN	GD	BA, HL	Parents DNA normal
Kelberman 2006	c.479delA	AN	AN	GD	BA, EA, GU, HL	Parents DNA normal
Bakrania 2007	c.480C>G	MI	MI		GI, GU	
Kelberman 2006	C.480insA	MI	MI	GD	BA, EA, GU, MA	Parents DNA normal
<b>this study patient 5</b>	c.487ins2	AN	AN	GD	BA, GI, GU, MA, EN, SS	Parents DNA normal
Fantes 2003; Ragge 2005	c.529C>T	AN	AN	GD	GU, MA, SZ,	Parents DNA normal
Fantes 2003; Ragge 2005	c.529C>T	AN	AN	GD	HL, GU, MA, SZ	Parents DNA normal
Kelberman 2006	c.529C>T	AN	AN	GD	EA, GU	Parents DNA normal
<b>this study patient 6</b>	c.540C>G	MI	AN	MD	BA, GI, GU, SZ	Parents DNA normal
Schneider 2008; Zhou 2008	c.551delC	AN	MI	GD	BA, EA, GI, GU, SZ,	Mutation seen in affected sibling, unaffected mother (mosaicism proposed)
Schneider 2008; Zhou 2008	c.551delC	AN	AN	GD	BA, EA	Sibling of above
Ragge 2005	c.628delA	AN	AN	NOR	BA, SZ	Parents DNA normal
<b>this study patient 7</b>	c.937delI	AN	AN	GD	CV, SZ	Parents DNA normal
Ragge 2005	c.943del2	AN	AN	GD	BA	Parents DNA normal
<b>this study patient 8</b>	c.69ins3	AN	AN	GD	BA, EN, SS	Affected half-brother:mother's DNA normal
<b>Total</b>	<b>40</b>	AN=30 MI=5 O=2 NOR=3	AN=27 MI=9 O=2 NOR=2			
<b>GENE DELETION</b>						
Bakrania 2007	3' deletion	AN	AN	NOR		
Bakrania 2007	deletion	AN	CA	GD	BA, GU, SZ	
Bakrania 2007	deletion	MI, RD	MI, RD		GI	
Bakrania 2007	deletion	MI, RD	MI, RD	MD	SS	
Bakrania 2007	deletion	NOR	AN	GD	EA, GU	
Bakrania 2007	deletion	AN	MI, SC			
Williamson 2006	deletion	AN	AN		EA, GU, TEF	Parents DNA normal
Fantes 2003; Ragge 2005	deletion	AN	AN	GD	BA, MA, SS	Parents DNA normal



Reference	Mutation	R Eye	L Eye	Delay	Other	Family History
<b>NONSENSE/FRAMESHIFT/TRUNCATION MUTATIONS</b>						
Kelberman 2008	deletion	AN	MI	GD	BA, CV, EN	
Guichet 2004	deletion	AN	AN		BA, EN, SS, GI	
<b>Total</b>	<b>10</b>	AN=7 MI=2 N=1	AN=5 MI=4 O=1			
<b>MISSENSE MUTATIONS</b>						
<b>this study patient 9</b>	c.131C>G (p.P44R)	NOR	AN	mild GD	BA, GU, HL	Parents DNA normal
Faivre 2006	c.138T>G (p.N46K)	AN	AN		BA	Affected sibling; mutation seen in unaffected mother(mosaicism proposed)
Williamson 2006	c.221G>C (p.R74P)	MI	MI		EA, TEF	
Sato 2007	c.224T>A (p.L75Q)	AN	NOR	NOR	EN	
Ragge 2005	c.290T>C (p.L97P)	MI, COL, SC	SC, AK	GD	MA, SZ	Parents DNA normal
Kelberman 2006	c.389G>C (p.G130A)	ONH	ONH		BA	Mutation also seen in unaffected father
Kelberman 2006	c.389G>C (p.G130A)	NOR	NOR	NOR		Father of above
Kelberman 2006	c.571G>A (p.A191T)	ONH	ONH		BA, EA	Mutation also seen in unaffected father
Kelberman 2006	c.571G>A (p.A191T)	NOR	NOR	NOR		Father of above
Wang 2008	c.695C>A (p.T232N)	MC, IH, PU	COL	NOR	BA, GU	Mutation also seen in affected father
Wang 2008	c.695C>A (p.T232N)	CA, COL	NOR	NOR		Father of above
<b>this study patient 10</b>	c.859G>C (p.A287P)	NOR	MI	NOR		
<b>Total</b>	<b>12</b>	AN=2 MI=2O=4 NOR=4	AN=2 MI=2O=4 NOR=4			

AN, Anophthalmia; MI, Microphthalmia; AK, Aphakia; ASD, Anterior segment dysgenesis; CA, Cataract; COL, Coloboma; CR, Choroidretinal dystrophy; ET, Esotropia; GL, Glaucoma; IH, Iris hypoplasia; MC, Microcornea; MY, High myopia; ONH, Optic nerve hypoplasia; PU, Pupillary abnormality; RD, Retinal detachment; SC, Sclerocornea

BA, Brain anomalies; CV, Cardiovascular anomalies; EA, Esophageal atresia; EN, Endocrinologic anomalies; FD, Feeding disorder/reflux; GD, Global delay; GU, Genitourinary anomalies; HL, hearing loss; MA, Muscle abnormality; MD, Motor delay; NOR, Normal; SS, Short stature; SZ, Seizures; VA, Vertebral anomaly