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Letter to the editor

A novel heterozygous *SOX2* mutation causing anophthalmia/microphthalmia with genital anomalies

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A B S T R A C T

Anophthalmia/microphthalmia is a rare developmental craniofacial defect, which recognizes a wide range of causes, including chromosomal abnormalities, single-gene mutations as well as environmental factors. Heterozygous mutations in the SOX2 gene are the most common monogenic form of anophthalmia/microphthalmia, as they are reported in up to 10–15% cases. Here, we describe a sporadic patient showing bilateral anophthalmia/microphthalmia and micropenis caused by a novel mutation (c.59_60insGG) in the *SOX2* gene. Morphological and endocrinological evaluations excluded any anomaly of the hypothalamus-pituitary axis. Our finding supports the hypothesis that SOX2 is particularly prone to slipped-strand mispairing, which results in a high frequency of point deletions/insertions.

Anophthalmia/microphthalmia is a rare developmental craniofacial defect occurring in 2–3 per 10,000 population and consisting in the absence (i.e., anophthalmia) or the reduction in size (i.e., microphthalmia) of one or both eyes [1]. Anophthalmia/microphthalmia recognizes a wide range of causes, including chromosomal abnormalities, single-gene mutations as well as environmental factors [2]. Heterozygous mutations of the *SOX2* gene are the most common monogenic form of anophthalmia/microphthalmia, as they are reported in up to 10–15% cases [3,4]. *SOX2* is a single-exon gene encoding for a member of the SOX family of proteins, which belong to the class of transcriptional regulators with the high mobility group (HMG) domain [5]. Expression studies suggest a pivotal role for the *SOX2* gene in the developing central nervous system, in particular pituitary, forebrain and eye [6]. Furthermore, the occurrence of a wide range of extracephalic ancillary malformations in patients with *SOX2* mutations mirrors a possible role of this gene in the development of additional body structures, including gastrointestinal and urogenital systems [7]. We report an Italian patient with bilateral anophthalmia/microphthalmia and a novel mutation in the *SOX2* gene. Mutation type and position strengthen previous considerations on the mechanism underlying the *SOX2* molecular pathogenesis.

The propositus was a 6-month-old child, born to a 32-year-old Caucasian woman and her 33-year-old healthy husband. Parents were second cousins and family history was unremarkable. The patient had a dizygotic twin, who was healthy. Pregnancy was obtained by intracytoplasmic sperm injection after a 2-year-long period of infertility. Delivery was performed at 35 gestational week. Serologic tests for toxoplasmosis, herpes virus, rubella, and cytomegalovirus as well as triple serum marker screening were all negative. Birth length was 46 cm (50th centile), weight 2090 g (10th centile) and OFC 31 cm (10th–50th centile, all corrected for gestational age). At birth, clinical anophthalmia and severe microphthalmia were noted on the right side and left, respectively. The penis was slightly reduced in length. No other anomaly of the external genitalia was detected. Physical examination was otherwise unremarkable, and early psychomotor development was normal. At 6 months of life, the patient weighted 7050 g (25th centile), his length was 67 cm (25th–50th centile) and head circumference 41.5 cm (9th centile). Facial dysmorphism included marked reduction in size of the right eye and mild shrinking of the left one, depressed and widened nasal bridge, and frontal bossing (Fig. 1A). There was also micropenis (penis length: 2 cm) with normally descended testes (Fig. 1B). Ophthalmological examination confirmed right anophthalmia and left microphthalmia with congenital cataract. Basal LH, FSH, testosterone, ACTH, cortisol, free T4, and TSH plasma levels were normal on repeated dosages. On magnetic resonance imaging, the right globe was virtually absent, while the left one was reduced in size with coloboma of the optic nerve (Fig. 1C). Hypothalamus-pituitary axis was morphologically normal (Fig. 1D). The study of the hippocampal region demonstrated bilateral dilatation of the temporal horns and hypoplasia of the hippocampus (Fig. 1E). Micropenis was successfully treated with three monthly injections of intramuscular testosterone.

After receiving informed consent, blood samples were obtained from the nuclear family. Genomic DNA was isolated using Promega Maxwell 16 purification kit (Promega Corporation, Madison, WI, USA). Overlapped PCR primers pairs were designed to amplify the coding region of the *SOX2* consisting of a single exon (amplicon 1 forward: 5′-AGTCCCGGCCGGGCCGAG-3′; amplicon 1 reverse: 5′-GG TAGCCCAGCTGGTCCTG-3′; amplicon 2 forward: 5′-GGCGTGAACCA GCGCATGG-3′; amplicon 2 reverse: 5′-GAGCGTACCGGGTTTTCTC-3′).

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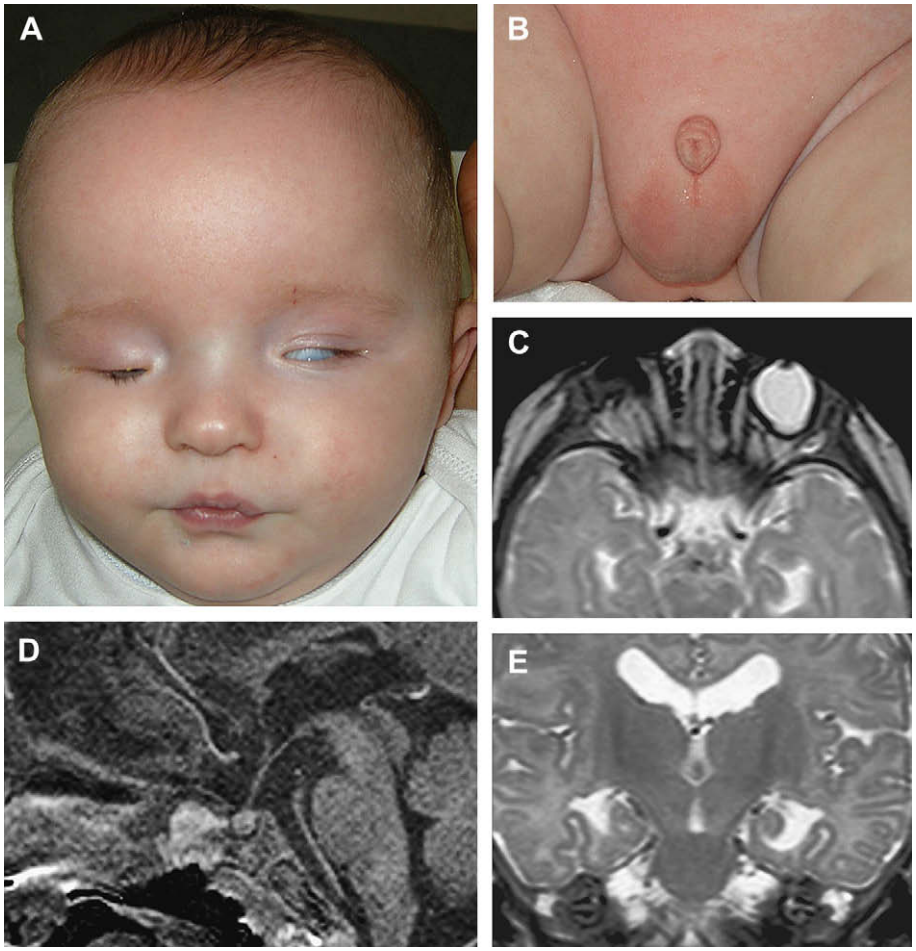

Fig. 1. (A,B) Clinical features of the patient at 6 months of age. (A) Note clinical anophthalmia on the right and severe microphthalmia on the left. Additional facial features include frontal bossing, and widened and depressed nasal bridge. (B) Micropenis with normally descendent testes. (C–E) Brain magnetic resonance imaging. (C) Axial view (T2 TSE weighted) illustrating right anophthalmia and left microphthalmia with colobomatous optic nerve. Right eye prosthesis is marked by an asterisk. (D) Sagittal view (T1 3D FFE weighted) showing absence of morphological anomalies of the hypothalamus-pituitary axis. (E) On coronal section (T2 TSE weighted), temporal horns are enlarged with relative hypoplasia of the hippocampus.

PCR and sequence reaction conditions are available on request. Direct sequencing of amplified fragments was carried out using the ABI Prism Big Dye Terminator v 1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an automated sequencer (ABI PRISM 310 DNA Sequencer). Sequences were analysed using a sequencing analysis software (V 5.2 Patch 2) and compared to the *SOX2* reference sequence (GenBank accession number: NM_003106).

Sequencing of the patient's DNA showed a heterozygous insertion of two guanines between nucleotides 59 and 60 (c.59_60insGG). The mutation was not detected in both parents, in the dizigotic twin (Fig. 2A), as well as in 200 control chromosomes. The insertion results in a frameshift mutation and introduces a premature termination signal 26 codons downstream. The predicted truncated protein contains 45 aminoacids and only 19 of them were conserved. The identified mutation causes loss of 94% of the encoded protein (i.e., 298 aminoacids), including the HMG and the C-terminal transactivation domains, and part of the N-terminal domain.

In our patient, the concurrence of bilateral anophthalmia/microphthalmia and abnormal genitalia suggested perturbation of the *SOX2* function. Although parents were consanguineous suggesting recessive inheritance, we identified a novel heterozygous mutation in the *SOX2* gene. The pathogenic potential of the mutation was primarily suggested by the predicted effect at the protein level. To date, the *SOX2* mutation repertoire comprises whole gene deletions, as well as nine nonsense, 13 frameshift, seven missense and two 3′UTR mutations [6,8–10]. The identified pathogenic variant (c.59_60insGG) is novel and rise the number of *SOX2* point mutations to 32 (Fig. 2B). As *SOX2* is a single-exon gene, nonsense mediated mRNA decay cannot be invoked and a truncated protein is the predicted consequence of the premature stop codon. The most relevant active sites of the protein were completely abolished by the induced frameshift. Therefore, a loss-of-function effect is likely. This hypothesis is further supported by the lack of differential disease expression between patients with *SOX2* point mutations and those with whole gene deletions [8]. Interestingly, the insertion of a single guanine in position 60 (c.60insG) was described in a previous anophthalmia/microphthalmia patient [11]. In addition, more than 40% of the *SOX2* identified point mutations are insertions/deletions. Among them, 5 out of 13 are located within the first 70 nucleotides (Fig. 2B). In keeping with our findings and literature data, it could be hypothesized that the high amount of cytosines and guanines makes *SOX2* prone to specific mutational events, including slipped-strand mispairing [12], which appear more frequent in the N-terminal domain.

The involvement of male genital tract is a well known consequence of *SOX2* mutations. Recent evidence suggests that this feature might be sequential to hypothalamus-pituitary perturbation, as the