Genetic analysis of SOX2 and VSX2 genes in 27 Egyptian families with anophthalmia and microphthalmia

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Anophthalmia/microphthalmia (A/M) are major congenital anomalies of eye development with an estimated prevalence of 1 per 10,000 in live birth in developed countries. They could be isolated or as a part of syndrome when associated with other congenital abnormalities. Despite the identification of >20 disease-causing genes for isolated A/M, heterozygous mutations in sex determining regions Y-box 2 (SOX2) gene account for ~20% of all reported patients and visual system homeobox 2 (VSX2) could account for ~15% of consanguineous patients from the Middle East. That is why we initially focused to screen for SOX2 and VSX2 mutations in 27 Egyptian patients with A/M. Our cohort included 12 females and 15 males (Table S1). Positive consanguinity was evident in 16 families (59.5%). Nine of the patients (33.3%) had bilateral anophthalmia, 14 (51.9%) had bilateral microphthalmia, 3 (11.1%) had unilateral anophthalmia, and another one (3.7%) showed unilateral microphthalmia. Other ophthalmological findings included bilateral total retinal detachment in seven patients (25.9%), cataract in five patients (18.5%), coloboma in five patients (18.5%), orbital cyst in three patients (11.1%), and persistent hyperplastic primary vitreous in two patients (7.4%). This research was reviewed and approved by the Research Ethics of the National Research Centre according to "World Medical Association Declaration of Helsinki" and written informed consents were obtained. Genomic DNA was extracted from peripheral blood leukocytes of the patients and their parents using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The single SOX2 exon 4-6 was polymerase chain reaction (PCR) amplification using two overlapping fragments, while the five sets of primers were used for amplification of VSX2 coding sequences. PCR products were purified and sequenced using the standard techniques (Big Dye Terminator, Applied Biosystems, Foster City, CA). Our study revealed a previously reported heterozygous nonsense mutation c.480G>C (p.Y160*) of SOX2 (Figure 1) in one female patient (3.7%). This mutation was found in the transactivation domain and it is predicted to result in the formation of a truncated SOX2 protein. This girl (patient 1) had bilateral anophthalmia at birth and she was the first baby born to a healthy nonconsanguineous parents. On examination at the age of 10 months, her weight was 10,000 g (mean), her length was 67 cm (~2.7 SD), and her occipitofrontal head circumference was 46.5 (~0.6 SD). She had subtle craniofacial dysmorphism in the form of high forehead, sparse eyebrows, and low-set ears (Figure 1). Left choanal atresia was noticed. Her muscle tone was normal. Her developmental milestones were within normal. She had no history of seizures and evidence of hearing loss. Brain MRI showed absent globes on both sides and no evidence of optic nerves development, cavum septum pellucidum, thinning of corpus callosum with suspected herniation of the meningeal sac, and brain parenchyma into the left nasal fossa (Figure 1). It is noteworthy to mention that this is the third time this heterozygous nonsense mutation c.480G>C (p.Y160*) of the SOX2 gene has been reported in the literature. In comparison, one of the previously reported patients presented with severe right microphthalmia and left anophthalmia, micropenis and cryptorchidism5 and diagnosed as SOX2-anophthalmia syndrome, while the other patient had bilateral anophthalmia and Dandy–Walker malformation. Since this heterozygous nonsense mutation c.480G>C (p.Y160*) of SOX2 was observed in isolated and SOX2-anophthalmia syndrome and given the observed gonadotropin deficiency with SOX2 patients, even without anophthalmia, we could suggest a continuum spectrum named SOX2 spectrum. To our knowledge, the herniation of the meningeal sac and brain parenchyma into the left nasal fossa found in our patient has not been observed earlier.

While Schneider et al.4 reported that patients with missense mutations had milder ocular phenotypes than those with nonsense or frameshift mutations, Suzuki et al. denied this observation and the genotype–phenotype correlation. Nevertheless, Zhou et al.7 speculated that untranslated region mutations of SOX2 are associated with severe eye development and less likely associated with neurological manifestations. Our patient had bilateral clinical anophthalmia caused by nonsense mutation, thus supporting Schneider et al. notion.6 The reported familial patients with SOX2 mutations despite apparently normal parents highlighted the importance of careful analysis of parental
samples to exclude mosaicism. In keeping with most of the previously documented cases, this mutation was not found in the parents. Therefore, it is considered de novo.

Screening of VSX2 gene showed a previously reported homozygous mutation c.371-1G>A in the acceptor splice site of exon 1 in one male patient (Figure 1), thereby preventing splicing of the second exon in VSX2 gene which led to a nonfunctional VSX2 protein. Parents were heterozygous for the VSX2 mutation supporting the recessive nature of VSX2 gene mutations. The 16-month-old boy (patient 11) had bilateral microphthalmia more pronounced in the left eye. He had no craniofacial dysmorphism and a thorough clinical evaluation did not identify any associated congenital anomalies. His developmental milestones were mildly delayed (head support at 5 months, sitting at 8 months, stood with support 15 months, and he did not walk yet). Transpalpebral ultrasonography showed 12 mm length axial, membranous floaters in the vitreous, undetected lens and optic nerve in the left side versus 16 mm axial length with opaque lens but normal vitreous, retina, and optic nerve in the right side. Flash and pattern visual evoked potential of both sides showed marked reduced wave amplitudes and significant reduced responses of sides with marked impairment in the left eye.

This mutation (c.371-1G>A) was previously reported in a Syrian Jewish A/M patient in association with iris coloboma. Overall, in A/M patients caused by VSX2 mutation, coloboma of the iris is described in ~35% of cases and retinal detachment in 20% of cases. We neither noticed iris coloboma nor retinal detachment in patient 2, although cataract and hypoplastic optic nerve were observed. These associated eye anomalies have also been noted in a small number of individuals with VSX2 mutation. Extraocular features associated with VSX2 mutation are not common and presented as single cases of developmental delay with behavioral problems, autism, cryptorchidism, ovarian defects, or hearing impairment. Mild developmental delay was just observed in our patient.

The identification of VSX2 mutation in this study and in a previously reported Egyptian patient, although very rare, underscores the importance of screening of this gene in consanguineous families in our population and the Middle East.

Figure 1. (A) Pedigree of patient 1. (B) General appearance of patient 1 at the age of 1 month, showing high forehead, sparse eyebrows, and low-set ears. Brain MRI of patient 1 at the age of 10 months showing (C) cavum septum pellucidum, (D) absent globes on both sides with suspected herniation of the meningeal sac and brain parenchyma into the left nasal fossa, and (E) thinning of corpus callosum. Sequencing electrophoregram showing (F) normal mother and (G) the heterozygous SOX2 mutation, c.480G>C. (p.Y160*). (H) Pedigree of patient 11. Sequencing electrophoregram showing (I, J) heterozygous mother and father and (K) homozygous VSX2 mutation c.371-1G>A in the acceptor splice site of exon 1 in patient 11. The arrows indicate the site of mutations.
In addition, a number of single-nucleotide polymorphisms (SNPs) were detected. These sequence variations were scattered in exons 1, 3, 5. The synonymous variants were as follows: c.471 C>T (p.S157S, rs35435463), c.831G>A (p.L277L, rs62006815), and c.294C>T (p.S98S, rs770121550) in nine, two, and five patients, respectively. In addition, a nonsynonymous polymorphism c.871G>A (p.D291N, rs75395981) was found in two affected individuals. Furthermore, a novel variant c.477 G>A (p.Q159Q) was identified in 1 patient. In contrast, no SOX2 synonymous polymorphism observed in our patient’s group.

In conclusion, targeted sequencing for SOX2 and VSX2 identified the etiology in two patients (7.4%) and this is the first report of SOX2 mutation from Egypt.

**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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