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Case Report

A Case of Bilateral Microphthalmia and Extensive Colobomas of the Globes Associated with a Likely Pathogenic Homozygous *SIX6* Variant

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Abstract
Colobomas of the globe and microphthalmia are congenital conditions that can strongly affect vision. Etiologies are varied and include embryonic and hereditary origins. We report what is, to the best of our knowledge, the first case of a *SIX6* gene pathogenic variant associated with a phenotype of both bilateral microphthalmia and extensive colobomas of the globes. A 3-week-old boy presented with bilateral microphthalmia and iris, optic nerve, and chorioretinal colobomas. Genetic analysis was performed on a panel of 78 genes (microphthalmia, anophthalmia, and coloboma panel), and a homozygous likely pathogenic variant was identified in the *SIX6* gene, resulting in the loss of the initiator methionine. Thus, our report expands the phenotypic spectrum of *SIX6*-related disorders.

Introduction

Colobomas of the uveal tissue, retina, and/or optic nerve are relatively rare congenital conditions caused by incomplete fusion of the optic fissure [1]. They result in missing tissue,

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which can lead to vision loss and other complications, depending on the structures affected [1, 2]. Potential etiologies are varied, and multiple genetic pathways are involved [1].

Colobomas can be accompanied by microphthalmia. This microphthalmia, anophthalmia, and coloboma (MAC) spectrum has been studied in an attempt to identify the genes responsible for this phenotype [3].

One of the identified candidate genes is *SIX6*, a member of the *SIX/sine oculis* homeobox gene family [3]. *SIX6* plays a key role in the development of the retina, optic nerve, and hypothalamic and pituitary regions [4, 5]. In the investigation performed by Aijaz et al. [6] in a cohort of 173 patients with MAC, no evidence was found of *SIX6* pathogenic variants underlying these malformations. Nonetheless, there are reports of recessive and dominant pathogenic variants of the *SIX6* gene in association with the MAC spectrum, but none of which describe a phenotype comprising both microphthalmia and coloboma [3]. To the best of our knowledge, we hereby report the first case of bilateral microphthalmia and extensive colobomas of the globes in the setting of a genetic panel positive for a likely pathogenic homozygous *SIX6* variant.

Case Report

A 3-week-old boy, born at 36 weeks, was referred to our Pediatric Ophthalmology service for assessment of eye malformation. Family history was negative other than parents being first cousins. The first examination revealed bilateral (OU) microphthalmia and inferior iris, optic nerve, and chorioretinal colobomas (as shown in Fig. 1; photos taken under general anesthesia at age 4 years). Microphthalmia was confirmed at age 3 months by corneal diameters of 8.0 × 8.5 mm OD and 8.5 × 8.5 mm OS; of note, estimated axial lengths were of 19 mm OD and 17.5 mm OS on MRI done at age 1 year 4 months (as shown in Fig. 2). In order to rule out a potential multiorgan genetic syndrome, abdominal, spinal, and cerebral ultrasounds were performed, which came back normal. The patient developed horizontal nystagmus a few weeks later and showed fixation preference with OS. A large-angle OD esotropia also developed. Examination under general anesthesia at age 3 months confirmed the structural abnormalities described. The intraocular pressure was considered close to/within normal limits (under general anesthesia, using Tono-Pen: 10 mm Hg OD and 10 mm Hg OS at 3 months of age; 11 mm Hg OD and 13 mm Hg OS at 2 years of age), and cycloplegic refraction using retinoscopy revealed high myopia and astigmatism OU (at 3 months of age: OD −19.00 +4.25 ×20° and OS −13.00 +3.25 ×160°). Glasses with the full cycloplegic correction were prescribed, and daily patching of the left eye was prescribed in an attempt to treat concomitant amblyopia OS. Visual acuity was not measurable with Teller acuity cards (no interest from the patient). At 1 year of age, we could only objectively remark that the patient could fixate with both OD and OS but only seemed to follow with OS. Cerebral and orbital MRI imaging, shown in Figure 2, revealed slight globe size asymmetry (OD > OS), optic nerve coloboma OU, a cyst continuous with the optic nerve coloboma OD, ectasia of the optic nerve sheaths (worse OD), and slightly smaller optic nerves. The intracranial structures looked normal. Endocrinological investigation was also normal, and the patient was referred to genetics. Amblyopia treatment was unsuccessful, and subsequent examination under general anesthesia allowed updating the patient's spectacles at ages 16 months, 27 months, and 4 years. The IOP remained normal, and no retinal detachment was seen. The patient underwent strabismus surgery.

Genetic analysis was performed on a panel of 78 genes (MAC spectrum panel): *ABCB6*, *ACTB*, *ALDH1A3*, *ATOH7*, *BCOR*, *BMP4*, *BMP7*, *C12orf57*, *CC2D2A*, *CHD7*, *CLDN19*, *COL4A1*, *CRYBA4*, *CYP1B1*, *ERCC1*, *ERCC2*, *ERCC5*, *ERCC6*, *FOXC1*, *FOXE3*, *FOXL2*, *FRAS1*, *FREM1*, *FREM2*, *GDF3*, *GDF6*, *GJA1*, *GLI2*, *GRIP1*, *HCCS*, *HESX1*, *HMX1*, *IGBP1*, *IKBKG*, *LRP5*, *MAB21L2*, *MFRP*, *MITF*,

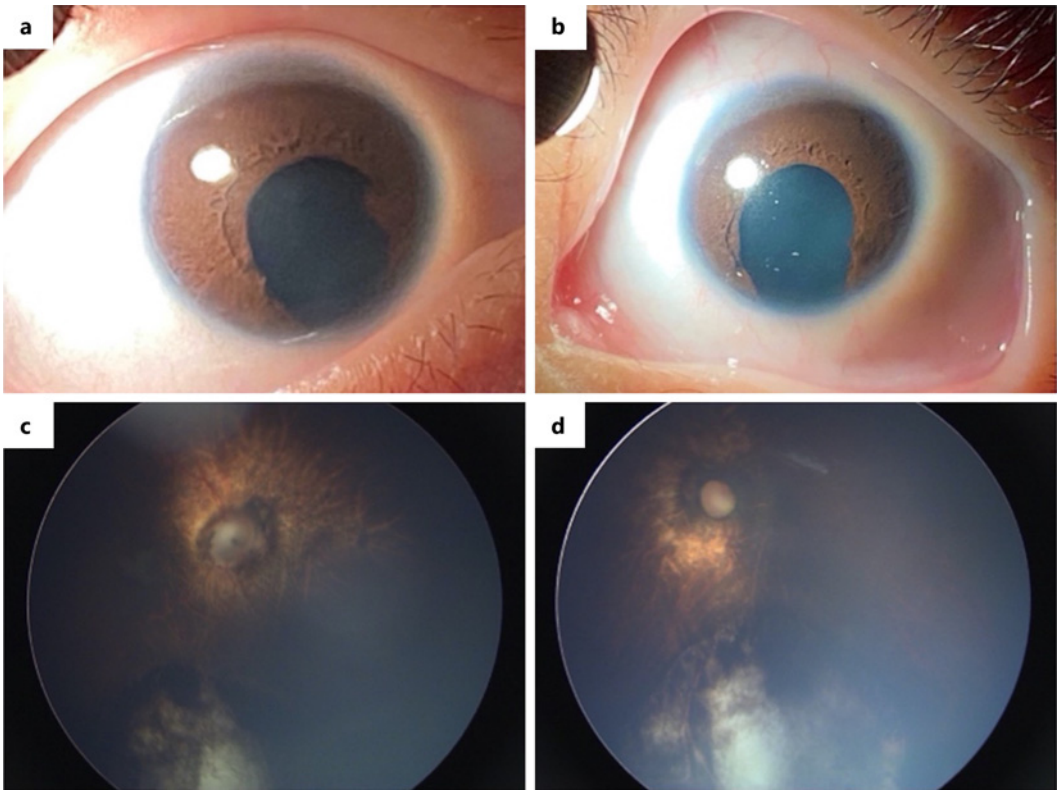

Fig. 1. Photographs taken under general anesthesia examination (patient aged 4 years). Inferior iris coloboma of the right eye (**a**) and left eye (**b**). Fundus photography shows inferior optic nerve and chorioretinal colobomas of the right eye (**c**) and left eye (**d**).

NAA10, *NDP*, *NHS*, *OCRL*, *OTX2*, *PAX2*, *PAX6*, *PIGL*, *PITX2*, *PITX3*, *PORCN*, *PQBP1*, *PRSS56*, *PXDN*, *RAB18*, *RAB3GAP1*, *RAB3GAP2*, *RARB*, *RAX*, *RBP4*, *RPGRIP1L*, *SALL2*, *SEMA3E*, *SHH*, *SIX3*, *SIX6*, *SMOC1*, *SOX2*, *SRD5A3*, *STRA6*, *TBC1D20*, *TENM3*, *TFAP2A*, *TGIF1*, *TMEM67*, *VAX1*, *VPS13B*, *VSX2*, *YAP1*, *ZIC2*. A likely pathogenic variant was identified in the *SIX6* gene (homozygous, c.1A>G (p.?)), consistent with a molecular diagnosis of a *SIX6*-related condition of autosomal recessive inheritance. This variant NM_007374.2:c.1A>G (p.?) is predicted to abolish the initiator methionine, resulting in a loss of expression of the protein product of the *SIX6* gene due to a lack of translation. No pathogenic variants were found in the analysis of the other genes, other than a heterozygous variant of unknown significance in the gene *RAB3GAP2* (NM_012414.4: c.4060A>G, p.Ile1354Val). Being a single heterozygous variant for a recessive gene, especially in a family with consanguineous parents, this variant was not considered to be involved in the patient's phenotype. Indeed, this variant changes a branched-chain amino acid for another, the isoleucine is not a conserved amino acid (notably, elephants have a valine at that position), and the variant is found quite frequently (as high as 1% frequency in individuals of African origin are carriers) in the gnomAD population genetics database (v3.1.2) [7].

Conclusion

Microphthalmia describes the presence of a small eye within the orbit and comprises a wide array of etiologies, including chromosomal, monogenic, and environmental [8]. The

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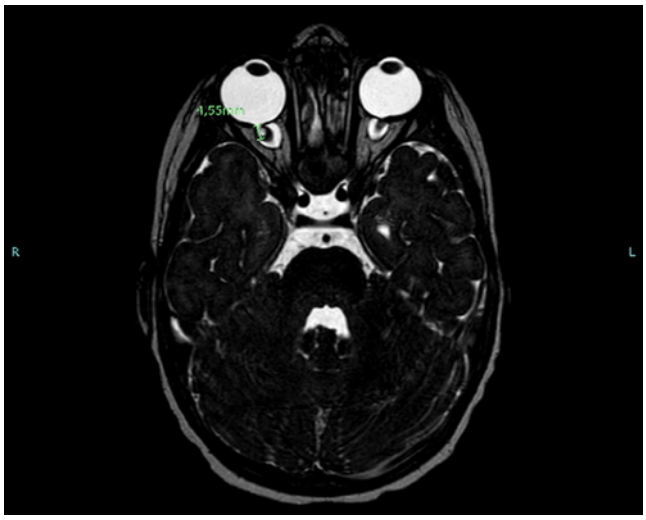

Fig. 2. Brain and orbit MRI C-, T2 (patient age: 1 year 4 months), revealing several orbital anomalies: globe size asymmetry (OD > OS), optic nerve coloboma OU, a cyst continuous with the optic nerve coloboma OD (as shown by the 4.5 mm measurement), the optic nerve coloboma OS presenting as a small deformity near the optic nerve insertion, ectasia of the optic nerve sheaths (worse OD), and bilaterally slightly smaller optic nerves. The chiasm and optic tracts appear within inferior normal limits. The intracranial structures appear normal for the patient's age.

study by Reis and Semina [3] on the conserved genetic pathways associated with the MAC spectrum reviewed and summarized the pertinent literature regarding *SIX6* in MAC conditions; "both recessive and dominant mutations in *SIX6* have been reported in MAC conditions."

Gallardo et al. [9] performed variant analysis of the *SIX6* gene in 73 patients with anophthalmia/microphthalmia, identifying three relatively frequent polymorphisms and a single heterozygous potential causative missense variant (c.493A>G; T165A) in a proband with bilateral microphthalmia and cataract; of note, they could not confirm this *T165A* variant as causative for the phenotype. Aldahmesh et al. [10] identified a homozygous truncating *SIX6* pathogenic variant in two siblings presenting with bilateral microphthalmia, associated with anterior and posterior segment dysgenesis (without evidence of coloboma). The authors determined that such evidence orients *SIX6* as a disease gene for microphthalmia but acknowledge that the supporting evidence is circumstantial at best [10].

Yariz et al. [11] reported the case of three children of a consanguineous family presenting with optic disc anomalies, macular atrophy, and colobomas of the iris and chorioretina (without microphthalmia or cataract) in the setting of a *SIX6* pathogenic variant. In a large cohort study, Aijaz et al. [6] hypothesized that *SIX6* pathogenic variants may underlie MAC phenotypes and searched for *SIX6* variants in a group of 173 patients with various combinations of unilateral or bilateral anophthalmia, microphthalmia, and colobomas. The two exons of their *SIX6* gene were amplified, sequenced, and compared to a control group of healthy patients. They found six different single-nucleotide substitutions in the *SIX6* gene, of which three lead to an amino acid change. However, after comparison with the control groups, the authors concluded that these changes do not predispose toward congenital eye malformations [6].

The current literature emphasizes the need for further research on microphthalmia and coloboma phenotypes and their possible association with *SIX6* gene variants and other genes involved in eye development. There may be an association between a phenotype of both bilateral microphthalmia and iris, optic nerve, and chorioretinal colobomas with an autosomal recessive homozygous pathogenic variant in the *SIX6* gene.

Statement of Ethics

This retrospective review of patient data did not require ethical approval in accordance with local/national guidelines. Written informed consent to publish personal information,

case details, and images has been obtained from the patient's parent (legal tutor) since the child is too young to provide his own consent. Institutional approval is not required to publish the case details according to the authors' institution guidelines.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Eileen Javidi: literature review; preparation of original draft; writing of original manuscript; and manuscript editing and revision. Dr. Simon Javidi: treated the subject and collected the clinical data; writing of original manuscript; and manuscript editing and revision. Dr. Philippe M. Campeau: critical feedback and manuscript editing and revision. Dr. Luis H. Ospina: treated the subject and collected the clinical data; critical feedback; and manuscript editing and revision. All the authors gave their approval for submission of the final version and agreed upon the journal to which this article was submitted.

Data Availability Statement

All data analyzed in the course of this study have been included in this article. Further inquiries can be addressed to the corresponding author.

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