

## Case Report

# Bilateral anophthalmia and clitoris and labia minora agenesis. A case report

Viorica Radoi<sup>1,2</sup>, Delia Ileana Sabau, Radu Ursu<sup>1,2</sup>, Laurentiu Bohiltea<sup>1,2</sup>, Oana Toader<sup>1,2</sup>,  
Lucian Pop<sup>2</sup>

### Abstract

<sup>1</sup>“Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

<sup>2</sup>“Alessandrescu-Rusescu” National Institute

\*Corresponding Author Email:  
[drdeliasabau@hotmail.com](mailto:drdeliasabau@hotmail.com)

Complete bilateral anophthalmia is the congenital absence of both eyes. Ambiguous genitalia is the condition in which the patient's genitalia are incompletely developed or the baby has characteristics of both sexes. Pathogenic variants in SOX2 gene are associated with syndromic microphthalmia, type 3 (OMIM: 206900), with autosomal dominant inheritance pattern and with AEG syndrome (OMIM 600992). We report the case of a new born baby girl with bilateral anophthalmia and clitoris and labia minora agenesis, which originated from in vitro fertilization (IVF). Sequential genetic testing, QF-PCR, microarray and WES have been performed and DNA was obtained according to standard procedures. Whole exome sequencing (WES) identified a mutation in SOX2 (OMIM: 184429) gene, a heterozygous pathogenic variant, NM\_003106.4: c.371dupA p.(Tyr125Valfs\*14), highlighting the importance of this particular gene in embryogenesis, sexualization and the development of the visual function. It explains the clinical diagnosis of the patient. We have concluded that this particular mutation found in the SOX2 gene could be correlated to the pathological findings, although it was not reported before in the clinical databases. Furthermore, a prenatal testing algorithm would be very helpful. Additionally, we want to highlight the importance of a multidisciplinary team when diagnosing and managing the follow-up of a fetus with multiple malformation such as this particular case.

**Keywords:** Bilateral anophthalmia, Clitoris agenesis, Labia minora agenesis, SOX2, Syndromic

## INTRODUCTION

Complete bilateral anophthalmia is the congenital absence of both eyes. It is a rare disease often associated with other malformations such as urogenital or digestive. Ambiguous genitalia is the condition in which the patient's genitalia are incompletely developed or the baby has characteristics of both sexes.

Anophthalmia has an estimated rate of incidence of around 1 in 20 000 live births, clitoris and labia minora agenesis are rare conditions, with only few cases reported in medical literature.

SOX2 gene is an intronless gene that encodes a member of the SRY-related HMG-box (SOX) family of transcription factors involved in the regulation of embryonic development and in the determination of cell fate. The single exon gene is also expressed in the prospective neural plate, peripheral nervous system, nasal and otic placodes, bronchi, endoderm of the esophagus, and the germ cells of both sexes where it promotes normal sexual development. The product of this gene is required for stem-cell maintenance in the central

nervous system, and regulates gene expression in the stomach. Mutations in this gene have been associated with optic nerve hypoplasia and with syndromic microphthalmia or anophthalmia, ocular coloboma, hearing loss, hypogonadotropic hypogonadism and brain anomalies.

Next generation sequencing is an increasingly used tool for comprehensive prenatal genetic diagnostic. There is a wide range of high resolution genetic tests which can confirm genetic defects that are responsible for congenital anomalies evident on a prenatal ultrasound. Whole exome sequencing (WES) for prenatal diagnosis has a reported diagnostic yield of 6.2%–57% and it is a very useful tool to identify patients with a high likelihood of genetic mutations in cases with abnormal fetal ultrasound. Candidates for WES for fetal diagnosis had a normal karyotype and normal microarray with at least one of the following: parental consanguinity, large regions of homozygosity on fetal microarray, or high likelihood of single gene disorder based on ultrasound findings.

With the use of both karyotype and microarray, a genetic diagnosis is found in 6-10% of cases with fetal anomalies (Alamillo, 2015; Wapner, 2012). While many fetal anomalies will have no identifiable genetic cause, WES can improve the diagnostic rate in cases with normal microarray.

We report the case of a caucasian new born baby girl with bilateral anophthalmia and clitoris and labia minora agenesis, which originated from in vitro fertilization (IVF). The baby was born by natural vaginal birth, with a weight of 2950 grams. Anophthalmia was first noticed at 27 weeks of gestation, as this was the patient's first scan in pregnancy. A small orbit with absence of the lens was noted, signs that are consistent with a diagnosis of anophthalmia. Invasive testing and termination of pregnancy were discussed and declined by the family on religious grounds.

## MATERIAL AND METHODS

The parents of the patient agreed for collection of a cord blood sample at delivery for genetic testing. Sequential genetic testing, QF-PCR, microarray and WES have been performed in order to diagnose the newborn, following DNA extraction with quantitative and qualitative evaluation of the DNA obtained according to standard procedures. Capture and enrichment of exonic regions and flanking intronic areas of genes contained in the SureSelect Exome v6 (>20,000 genes) sequencing panel with the Agilent technology™. Paired-end massive sequencing with the NovaSeq™ (Illumina) sequencer.

Identification of the variants of interest located in coding regions and flanking intronic region (+/-10 bp), in regard to the reference genome (hg19) after filtering, according to specific quality criteria: call quality>20, coverage>10x, Genotype Quality>20 and Allele

fraction>20. Annotation, filtering and identification of variants by RefLabDB (database and in house pipeline) and Ingenuity Variant Analysis™ (QIAGEN). Analysis of variants using the data provided by the annotations and with the support of Alamut Visual™ (Interactive Biosoftware). The nomenclature used to define the variants follows the criteria of the Human Genome Variation Society (HGVS) (<http://www.HGVS.org/varnomen>).

Classification of variants based on the recommendations of the American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015) and Association for Clinical Genomic Science (ACGS) (Ellard et al., 2020).

## RESULTS

Sequential genetic testing (QF-PCR, microarray, WES) has been performed with Whole exome sequencing (WES) identifying a mutation in SOX2 (OMIM: 184429) gene, highlighting the importance of this particular gene in embryogenesis, sexualization and the development of the visual function.

The result of the genetic analysis identified the presence of a heterozygous pathogenic variant in SOX2 - NM\_003106.4:c.371dupA p. (Tyr125Valfs\*14). It explains the clinical diagnosis of the patient.

The detected SOX2 variant c.371dupA p. (Tyr125Valfs\*14) is a duplication/insertion of one nucleotide that causes a frameshift at position 125 of the protein, predicting an amino acid change from Tyrosine to Valine and resulting in a premature STOP codon 14 amino acids downstream. The protein will have 138 residues, being the native protein composed of 317. It is neither described in the clinical databases nor in the scientific bibliography consulted on the date of issue of the report. It does not appear neither in the dbSNP database nor in the gnomAD population frequency database.

## DISCUSSION

The SOX2 gene (OMIM: 184429) encodes the transcription factor SOX-2 protein. Pathogenic variants in this gene are associated with syndromic microphthalmia, type 3 (OMIM: 206900), with autosomal dominant inheritance pattern. Literature reviews show cases with SOX2 mutation manifesting Anophthalmia syndrome or AEG syndrome. Anophthalmia-Esophageal-Genital (AEG) syndrome (OMIM 600992) is an association of anophthalmia/microphthalmia, esophageal atresia with or without tracheoesophageal fistula, and urogenital anomalies—most commonly cryptorchidism, hypospadias and micropenis. The eye and genital phenotypes in both conditions appear indistinguishable (Kathleen et al., 2006).

Previously published data involving ambiguous genitalia and anophthalmia due to SOX 2 gene mutations have been reported mostly in male gender. Up to date we did find one case of this association in female gender (Menetrey et al., 2002). Our case report presented some distinctive features besides the eye malformation, the genital malformations, also the patient did not have the esophageal atresia.

Genetic counseling for the parents of the affected child and preconceptional management have to take into account that bilateral anophthalmia increases the probability of a genetic etiology, which is a fact proven by molecular testing. There is a 5% recurrence risk for future siblings due to germinal mosaicism, in case of unaffected parents. All form of Mendelian inheritance have been reported, but this particular case was autosomal dominant<sup>2</sup> (de novo mutation).

The further management for babies with anophthalmos is insertion of orbital expanders to encourage the eye socket to grow so that they can use prostheses from early childhood (Helen and Jane, 2017).

## CONCLUSIONS

We have concluded that this particular mutation found in the SOX2 gene could be correlated to the pathological findings, although it was not reported before in the clinical databases. Furthermore, a prenatal testing algorithm would be very helpful. First step, the carrier detection of the couple should be performed in families where a causative mutation is known and identified and ultrasounds in mid trimester that can visualize the eye and lens. Additionally, we want to highlight the importance of a multidisciplinary team when diagnosing and managing the follow-up of a fetus with multiple malformations such as this particular case.

## Declaration of Interests

The authors declare no competing interests.

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