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CASE REPORT

SEMILOBAR HOLOPROSENCEPHALY CAUSED BY A NOVEL AND DE NOVO *ZIC2* PATHOGENIC VARIANT

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ABSTRACT

Holoprosencephaly (HPE) is the most common embryonic forebrain developmental anomaly. It involves incomplete or absent division of the prosencephalon into two distinct cerebral hemispheres during the early stages of organogenesis. HPE is etiologically heterogeneous, and its clinical presentation is very variable. We report a case of a 7 month old female infant, diagnosed with non-syndromic semilobar holoprosencephaly, caused by a novel, *de novo* pathogenic variant in *ZIC2* - one of the most commonly mutated genes in non-syndromic HPE coding for the ZIC2 transcription factor. The patient presented with microcephaly, mild facial dysmorphic features, central hypotonia and spasticity on all four extremities. Ultrasound imaging demonstrated the absence of septum pellucidum, semilobar fusion of the hemispheres and mega cisterna magna and brain MRI with confirmed the diagnosis of HPE. Early diagnosis and management are important for the prevention and treatment of complications associated with this condition.

Keywords: holoprosencephaly, HPE, *ZIC2*, forebrain anomaly

INTRODUCTION

Holoprosencephaly (HPE) is the most common forebrain developmental anomaly in humans with an incidence rate of 1 in 250 spontaneous pregnancy losses and about 1.2 cases per 10,000-20,000 live births.[1,2,4] HPE results from an incomplete midline division of the prosencephalon and includes an extensive spectrum of intracranial and craniofacial anomalies.[1-3,5] A myriad of clinical manifestations may be present, which consist of neurologic impairment with global developmental delay, intellectual disability, seizures, brain anomalies and facial dysmorphic features. Neonates and infants with mild or pronounced facial dysmorphia may prompt early investigations, however, such features are not always present and the diagnosis is often delayed until the second year of life, when neuroimaging for developmental delay can reveal any anomalous brain morphology.

HPE classification is based on the degree of separation of the cerebral hemispheres and the severity of the clinical presentation. HPE Type 1 is characterized by lack of segmentation or complete fusion of the hemispheres (alobar HPE is the most severe form), characterized by the presence of a small single cerebral ventricle lacking interhemispheric division, corpus callosum, and olfactory bulbs. HPE Type 2 is characterized by partial segmentation of the brain (semilobar or lobar HPE; moderately severe). When semilobar, the frontoparietal lobes fail to separate; however, the interhemispheric fissure is present posteriorly, and the corpus callosum is either absent or hypoplastic. When lobar, a distinct interhemispheric fissure is present; however, some midline continuity of the cingulate gyrus persists. HPE Type 3 demonstrates almost complete segmentation (middle interhemispheric variant, also known as syntelencephaly, MIHV, least severe), with separation of the basal forebrain, anterior frontal lobes and occipital regions and failure to divide the posterior frontal and parietal regions of the cerebral hemispheres along the dorsal midline.[3-5,8,14]

The etiology of HPE is complex and includes both chromosomal and monogenic genetic causes as well as environmental factors, such as maternal type 2 diabetes, alcoholism and prenatal exposure to teratogenic drugs.[5-9,17]

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Numeric chromosomal anomalies are found in 25-50% of all HPE cases, and these are more likely to have additional syndromic features. The most common chromosome anomaly associated with HPE is trisomy 13 (about 40% of all HPE cases and 75% of all HPE cases due to chromosomal involvement), trisomy 18 and triploidy. Structural chromosome abnormalities found in HPE include 13q, and del(18p), del(7)(q36), dup(3)(p24-pter), del(2)(p21), and del(21)(q22.3). Copy number variants may account for up to 10% of all HPE cases.[3,4] The monogenic forms of HPE are cytogenetically normal, and include both syndromic (20-25% of all HPE cases) and non-syndromic forms of HPE.[3-5] The most commonly involved genes in syndromic autosomal dominantly inherited HPE are *CDON* (Steinfeld syndrome) and *FGFR1* (Kallman syndrome 2 and Hartsfield syndrome) and in syndromic autosomal recessively inherited HPE are CENPF (Stromme syndrome) and DHCR7 (Smith-Lemli-Opitz syndrome). The non-syndromic forms of monogenic HPE are most commonly caused by pathogenic variants in *SHH* (5-6 of non-syndromic HPE cases), *ZIC2* (about 5% of non-syndromic HPE cases) and *SIX3* (about 3% of non-syndromic HPE cases).[3-5]

CASE PRESENTATION

We present the case of a 7-month old Caucasian female infant, from a well monitored twin pregnancy, gemellus II with intra-uterine growth restriction. The morphology scan at 20 weeks' gestation revealed HPE and the parents were counselled about the clinical implications. The baby was delivered by caesarean section at 37 weeks' gestation (weight 2700 g, body length 48 cm and APGAR score 7 and 8 at 1 and 5 minutes). The clinical examination by a neonatologist noted microcephaly with a head circumference of 28 cm at birth. The infant had mild facial dysmorphic features including hypertelorism and a narrow nasal bridge (Figure 1A). There were no findings of ambiguous genitalia. Neurological examination revealed central hypotonia and spasticity on all four extremities. The rest of the physical examination findings were unremarkable.

Postnatal head ultrasonographic examination showed an absence of septum pellucidum, semilobar fusion of the hemispheres and mega cisterna magna (Figure 1B) and a brain MRI with FLAIR confirmed the diagnosis of HPE (Figure 2). Electroencephalography showed bihemispheric foci with a tendency for generalization (Figure 3) and transthoracic echocardiography revealed a subtle mid atrial septal defect, resulting in minimal L-D shunt and adequate kinetics of the heart. The abdominal ultrasonography was normal. Laboratory analyses revealed seropositivity for SARS-CoV-2, HSV and CMV, elevated alkaline phosphatase (303 U/L), non-significantly elevated arginine and C10 acylcarnitine and mild electrolyte disbalance (hypernatremia, hyperkalemia, hyperchloremia, hypercalcemia, hyperphosphatemia, hypermagnesemia).

Genomic testing using a panel of 456 genes associated with brain malformations identified a novel and *de novo* pathogenic *ZIC2* variant, initially interpreted as a variant of uncertain significance, and later reclassified as a likely pathogenic, according to the ACMG criteria.[24] This missense variant replaces amino acid cysteine at position 273 with tyrosine (Figure 4). The likely pathogenic missense *ZIC2* variant c.818G>A (p.Cys273Tyr) was not present in either of the two parents, and biological paternity and maternity was deduced from the analysis of ultra-rare genetic variants found in the proband. The variant has not

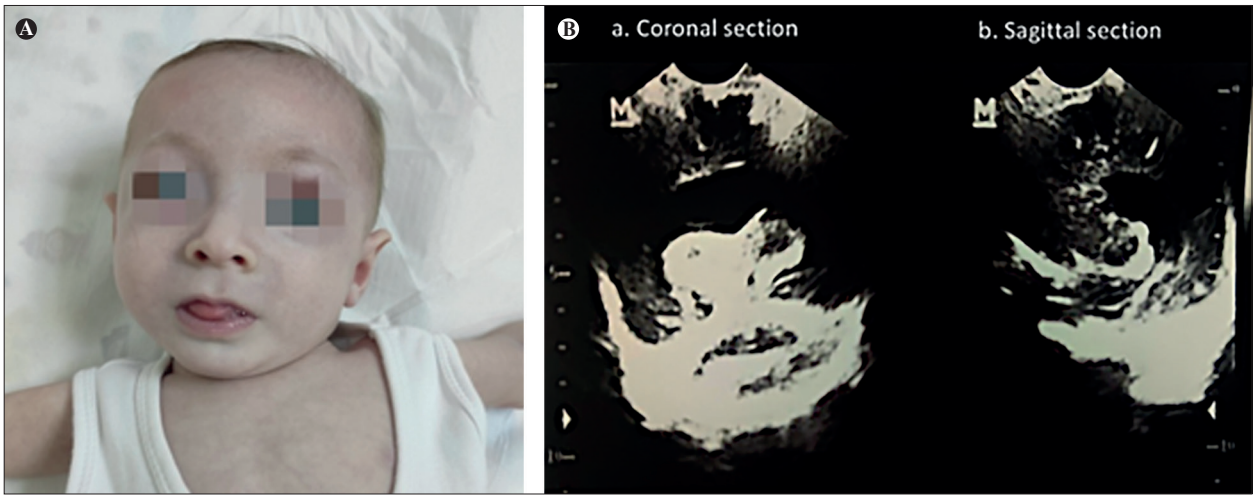

Figure 1. (A) Seven months old infant presenting with microcephaly and mild facial dysmorphic features, and (B) coronal (a) and sagittal (b) section of trans-fontanelle ultrasound examination revealing a single ventricle.

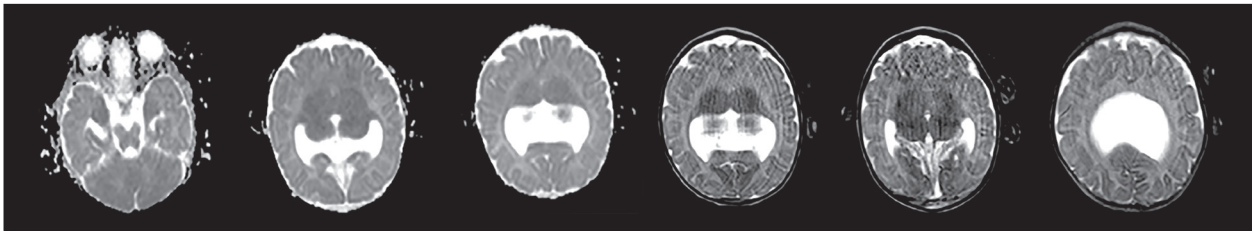

Figure 2. 1,5 T MRI showing mega cisterna magna, rudimentary anterior falx cerebri without formed fronto-parietal interhemispheric fissure, absence of septum pellucidum with agenesis of the corpus callosum, thinning of cerebrum, dysplastic unfused thalamus, rudimentary third brain ventricle, rudimentary temporal and occipital horns and cortical polymorphia with macrogyria and microgyria.

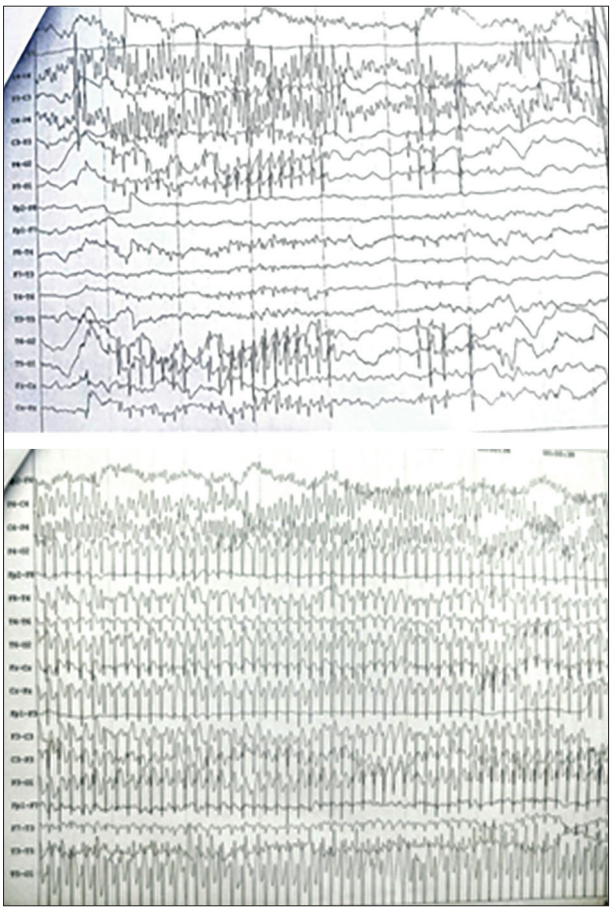

Figure 3. EEG demonstrating bihemispheric foci with a tendency for generalization.

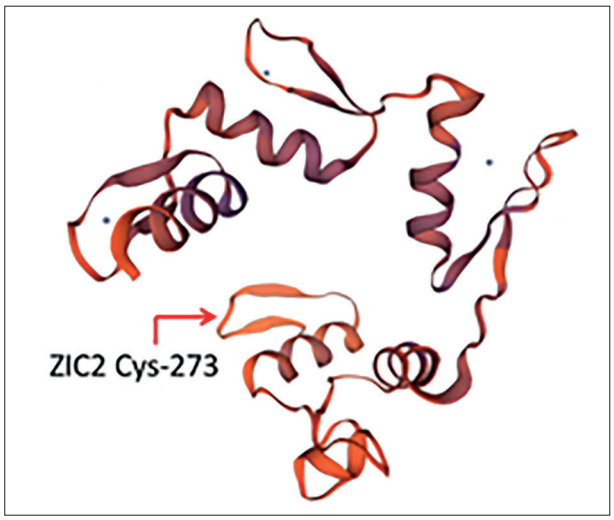

Figure 4. Molecular model of ZIC2 protein indicating the position of Cys-273.

been described before in patients with HPE and has no frequency in the databases of human genetic variation (ExAC, gnomAD). Bioinformatic tools (SIFT, PolyPhen-2, Align-GVGD) predict this to be likely disruptive. Interestingly, the genetic analysis identified another *ZIC2* variant (uncertain significance) c.1439C>T (p.Ser480Leu) in the proband, inherited from a healthy mother. The phase of the two variants could not be determined because the pathogenic variant occurred *de novo*. In addition, a number of additional variants of uncertain significance were identified (Table 1).

Table 1.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
ZIC2	c.818G>A (p.Cys273Tyr)	heterozygous	Likely Pathogenic
ZIC2	c.1439C>T (p.Ser480Leu)	heterozygous	Uncertain Significance
CTNNB1	c.1657A>G (p.Met553Val)	heterozygous	Uncertain Significance
PCLO	c.5293C>T (p.Pro1765Ser)	heterozygous	Uncertain Significance
PEX5	c.1814G>A (p.Ser605Asn)	heterozygous	Uncertain Significance
PTPN23	c.1603C>G (p.Gin535Glu)	heterozygous	Uncertain Significance

List of gene variants identified in the proband, including zygosity and variant interpretation.

DISCUSSION

Holoprosencephaly affects both genders equally and has been reported in different ethnic groups. The genetic etiology of HPE is complex, including both chromosomal and monogenic forms, resulting in either syndromic or isolated forms of HPE. Pathogenic variants in a dozen of genes have been reported to cause HPE, four of which (*SHH, ZIC2, SIX3, and TGIF*) are identified as more common and considered "major" causal genes.[20] Sonic Hedgehog (*SHH*) pathogenic variants are the most common single gene variants causing HPE and these patients are considered '*prototypical HPE patients'*. Approximately 10-30% of pathogenic *SHH* variants occur de novo.[5,21] The *SHH* gene affects mechanisms responsible for normal division of the prosencephalon during the embryogenesis. The associated phenotype is variable, ranging from an extremely severe form with craniofacial dysmorphia, including cyclopia and proboscis, to mild or non-penetrant forms without any notable deviations. The spectrum of the phenotypic presentation range from alobar HPE to microcephaly and hypoplasia of the pituitary gland in one family, and from HPE to an asymptomatic non-penetrant form in another family.[21] Sine Oculis Homeobox, Drosophila, Homolog of 3 (*SIX3*) codes for a transcription factor which controls the activity of genes involved in the embryonic development of the lens, retina, eye bulbs and forebrain. Variants in this gene are usually found in patients with severe HPE phenotypes including atelencephaly, complete absence of telencephalon or syntelencephaly.[5] Pathogenic variants in Transforming Growth Factor Beta-1 Induced Factor (*TGIF1*) are found in 1-2% of patients with HPE.[5,15,21] This gene is important for normal development of the forebrain and at least 13 *TGIF1* pathogenic variants have been found to cause nonsyndromic holoprosencephaly.[5]

Zinc Finger Protein of Cerebellum (*ZIC2*) is one of the genes most commonly associated with HPE. It plays a crucial role in the period of early embryogenesis, directly affecting the development of the dorsal telencephalon.[5,23] Almost 90% of patients with *ZIC2* HPE have structural brain anomalies that do not correlate with the extent of facial dysmorphia [10] therefore suggesting that *ZIC2* HPE is predominantly isolated, without syndromic or facial dysmorphic features common in HPE. These features are caused by chromosomal anomalies and pathogenic variants in other genes. The phenotype of *ZIC2* HPE patients can vary from mild forms to most severe HPE with microcephaly and cyclopia or synophthalmia below a proboscis. Less severely affected infants have microcephaly, and if the condition is complicated with hydrocephalus, the patient may have macrocephaly. HPE can be recognized in utero when brain morphology changes are seen during a prenatal ultrasound screening, as was the case with our patient. Postnatal diagnosis is usually prompted by neurological symptoms and typical facial dysmorphic features and established upon MRI examination. In our reported case, the child has an obvious structural brain anomaly and mild facial dysmorphia.

The most common clinical and often presenting problem in patients with HPC is severe neurological impairment [5,10]. Seizures are a frequent clinical feature in children with HPE and the therapeutic approach can be challenged by co-existing electrolyte imbalances. Depending on the severity of the condition, seizures can be difficult to control with antiepileptic drugs. In our case, the child was stabilized with 200 mg daily doses of levetiracetam. In other cases, when chorea is present, carbamazepine is the drug of choice.[16] The possibility of posterior pituitary insufficiency is high in patients with HPE and mild or no facial dysmorphia and the child was advised to be regularly followed up by a pediatric endocrinologist.

The dysmorphic facial features present in most patients with HPE include hypotelorism, midface hypoplasia with a flat nasal bridge, cleft lip and or/palate, and a single maxillary central incisor. The severity of facial dysmorphism is generally proportional with the degree of brain malformation and with the survival rate, except in patients with pathogenic *ZIC2* variants, as seen in our case.[5,20,23] Analysis of a large cohort of patients with pathogenic *ZIC2* variants demonstrated a common facial phenotype consisting of bitemporal narrowness and short nose with anteverted nares (not present in our case), flat nasal bridge and upslanting palpebral fissures (mildly present in our particular case), broad and deep philtrum, and disproportionally large ears, shown in Figure 1.[23]

ZIC2 variants occur *de novo* in approximately 72% of *ZIC2* HPE patients [5,10] and there are no reported families with *ZIC2* HPE where the pathogenic variant has been ascertained in more than 2 generations. The penetrance of *ZIC2* pathogenic variants is estimated to be very high, 96% for any manifestation and 90% for brain malformations.[10] The pathogenic *ZIC2* variant identified in our case occurred *de novo* (neither parent carried the pathogenic variant in blood DNA, paternity confirmed), and the family was counselled about the recurrence risk in the context of the possibility of gonadal mosaicism.

In conclusion, HPE is the most common brain malformation with a complex etiology that involves both genetic and environmental factors. Less severe forms, without complications, may have a long life span. Mildly affected children may live into adulthood, while severely affected children typically do not survive into early infancy. Although survival rates correlate with the severity of the brain malformation, there is significant variability within

each type of HPE. The group with the highest survival rate includes children with isolated HPE or with no associated chromosomal disease or syndrome. When HPE is diagnosed antenatally, careful genetic counselling in the context of variable clinical expressivity and reduced penetrance is essential to allow the family to arrive at their decisions. A multidisciplinary team approach to management is essential to maximize the prognosis of this complex condition.

CONSENT

Written informed consent was obtained from the patient for diagnostic genetic testing and publication of this case report with the accompanying images.

Conflict of interest

The authors declare that they have no competing interests.

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