

CASE REPORT

Middle interhemispheric variant of holoprosencephaly: First prenatal report of a ZIC2 missense mutation

Caroline Gounongbé¹  | Martina Marangoni²  | Vanessa Gouder de Beauregard³ |
Mélanie Delaunoy² | Patrice Jissendi⁴ | Marie Cassart^{1,4}  | Julie Désir^{2,4}

¹Department of Fetal Medicine, CHU Saint-Pierre, Brussels, Belgium

²Center of Human Genetics, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium

³Department of Pediatrics, CHU Saint-Pierre, Brussels, Belgium

⁴Department of Radiology, Hôpitaux Iris Sud and CHU Saint-Pierre, Brussels, Belgium

Correspondence

Caroline Gounongbé, Department of Fetal Medicine, CHU St Pierre, Rue Haute 322, 1000 Brussels, Belgium.

Email: caroline_gounongbe@stpierre-bru.be

Abstract

We present a case of a middle interhemispheric variant of antenatal discovery associated with a de novo missense variant (NM_007129.5: c.1109G>A p.(Cys370Tyr)) in the ZIC2 gene. Our case represents the first prenatal description of a ZIC2 missense mutation found in association with syntelencephaly.

KEYWORDS

middle interhemispheric variant of holoprosencephaly, missense mutation, syntelencephaly, ZIC2

1 | INTRODUCTION

Holoprosencephaly (HPE; MIM# 236100) is the most recurrent congenital brain malformation (1/10 000 live births), resulting from the incomplete midline division of the prosencephalon between 18th and 28th day of gestation and affecting the forebrain and the face.^{1,2} It is characterized by a wide clinical spectrum, ranging from severe HPE (alobar form: with a single cerebral ventricle and cyclopia) to clinically unaffected carriers. According to the degree of brain separation and whether the failure occurs ventrally or dorsally, two main classes of HPE can be distinguished: classic and middle interhemispheric variant (MIHV). In classic HPE, the lack of separation is most severe ventrally. This leads to a spectrum of classic HPE, of which alobar HPE is the most severe form, followed by semilobar and lobar forms (in which most of the

cerebral hemispheres are separated except at the ventral level of the frontal poles where the interhemispheric fissure remains absent).² A septopreoptic type, in which nonseparation is restricted to the subcallosal cortex and/or the ventral preoptic region, is also described in small case series.³ On the other hand, MIHV (otherwise known as syntelencephaly), firstly described in 1993,⁴ is characterized by the failure of division of posterior frontal and parietal regions of the cerebral hemispheres along the dorsal midline. Nevertheless, this condition entails the normal separation of the basal forebrain, anterior frontal lobes, and occipital regions.

Similarly to its wide clinical spectrum, HPE etiology is very heterogeneous.⁵⁻⁷ Environmental (consume of cumulative tobacco with alcohol, insulin-dependent maternal diabetes, retinoic acid and statins intakes) and infectious causes (toxoplasmosis, cytomegalovirus, syphilis, rubella)

Caroline Gounongbé and Martina Marangoni equally contributed to this work.

Marie Cassart and Julie Désir equally supervise this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2020 The Authors. *Clinical Case Reports* published by John Wiley & Sons Ltd.

are known to cause HPE. Moreover, chromosomal anomalies (numeric: trisomy 13, 18, triploidy; structural: deletions or duplications involving various regions of 13q, del(18p), del(7)(q36), dup(3)(p24-pter), del(2)(p21), del(21)(q22.3)) are responsible of the majority of HPE cases (up to 50%), while the remaining cases (including both syndromic or nonsyndromic HPE) are associated to pathogenic mutations. In nonchromosomal and nonsyndromic cases, HPE is usually considered to be inherited in an autosomal dominant mode.⁸⁻¹² In particular, fourteen genes have been implicated in nonsyndromic HPE (SHH, ZIC2, TGIF1, SIX3, CDON, DISP1, DLL1, FGF8, GLI2, FOXH1, GAS1, PTCH1, NODAL, TDGF1). In classic HPE, the most commonly mutated genes are SHH (12%) and ZIC2 (9%), which together account for ~85% of solved cases.^{9-11,13} ZIC2, located on chromosome 13q32, was firstly identified in patients with brain anomalies and harboring deletions involving the long arm of chromosome 13. It belongs to the zinc finger protein of the cerebellum family, encoding for a transcription factor that plays two distinct roles in the forebrain development. Interestingly, and conversely to the classic HPE genes, ZIC2 mutations have been found across the entire HPE phenotypic spectrum, including MIHV. In particular, just few mutations (splice variants, small deletions, and duplications) in ZIC2 have been reported in patients displaying MIHV to date. Herein, we describe a fetus presenting MIHV and harboring a de novo ZIC2 missense mutation.

2 | CLINICAL REPORT

We present the sixth pregnancy of a 39-year-old woman of Moroccan origin with no familial history of genetic diseases. Although a notion of second-degree consanguinity was known, the couple had already five healthy children. The mother was not ill during the pregnancy and did not take any medication, alcohol, or other toxic substances. She was protected for toxoplasmosis and rubella. Her serologies were negative for human immunodeficiency virus, hepatitis B and C viruses, and syphilis. The first trimester ultrasound performed at 13.6 weeks of amenorrhea appeared normal. Noninvasive prenatal testing was performed, and no chromosomal anomalies involving the chromosomes 13, 18, and 21 were found. The morphological ultrasound performed at 22 weeks was of poor quality and did not reveal any particular malformation. In addition, the diabetes screening was normal. During the 3rd trimester ultrasound, we observed the absence of a septal cavity with a fusion of the occipital horns of the lateral ventricles. In sagittal section, the corpus callosum seemed to be present in its anterior part but interrupted between the knee and the body. The anterior cerebral artery had a deviating

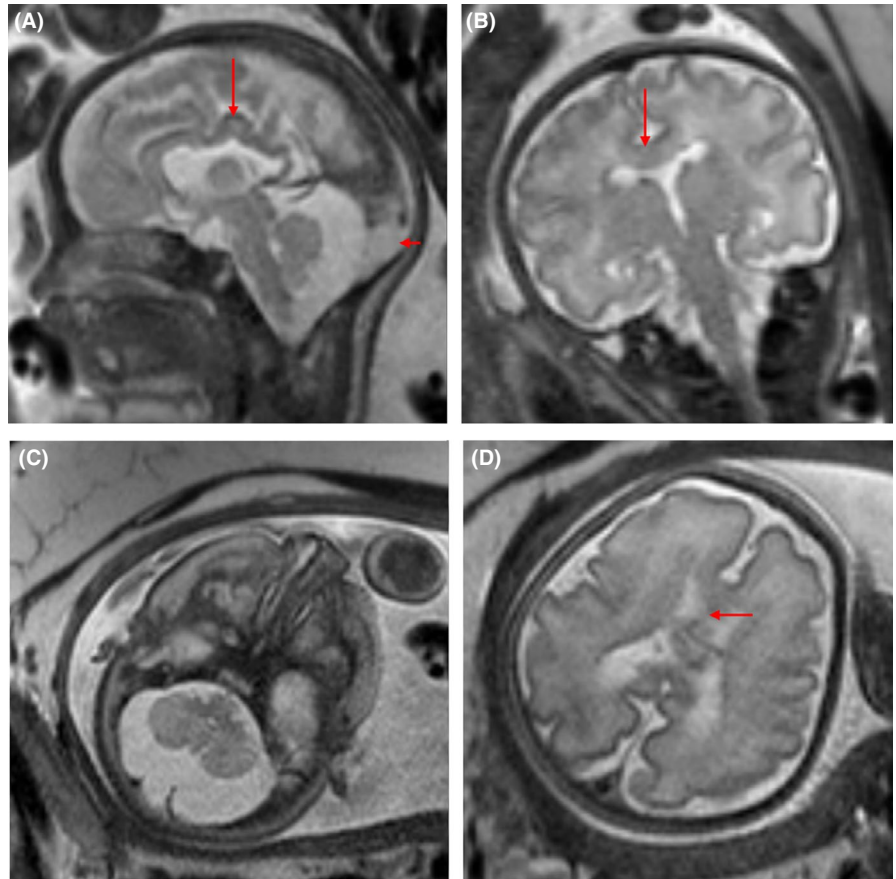
forward path, with an absent pericallosal artery. The lateral ventricles were moderately dilated (12 mm). The cerebellum was morphologically normal, and its measurement was less than the 3rd percentile according to Hadlock. No other anomalies were detected during the morphological examination.

We decided to carry out fetal cerebral magnetic resonance imaging (fMRI) and amniocentesis in order to perform array comparative genomic hybridization (array-CGH). The fMRI confirmed the septal agenesis and showed an interhemispheric parenchymal bridge connecting the frontal lobes, wrongly interpreted on ultrasound as the anterior part of the corpus callosum (Figure 1). It concluded in a complex midline malformation corresponding to syntelencephaly. Moreover, fMRI confirmed the cerebellar hypoplasia (Figure 1C) and depicted a posterior fossa arachnoid cyst (Figure 1A). On the other hand, as array-CGH showed no pathogenic chromosomal abnormalities, clinical exome sequencing was proposed to the couple. The analysis performed in duo (fetus and mother) revealed a probably de novo variant c.1109G>A p.(Cys370Tyr) in the exon 2 of the ZIC2 gene (NM_007129.5) (Figure 2A), predicted pathogenic and fitting with the brain phenotype. Sanger sequencing of both parents confirmed the de novo character (Figure 2B), allowing classifying the variant as probably pathogenic (Class IV). The couple decided to continue the pregnancy. At 35.3 weeks and after a spontaneous and premature labor, the mother gave birth to a girl of 2830 g, length of 46 cm, and head circumference of 36 cm. Her Apgar was 9-10-10 and, the pH at the cord was 7.23 and -3 excess base. Immediate neonatal adaptation was excellent. The neurological assessment on the third day of life was also reassuring, with a normal electroencephalogram and no dysmorphism. Cerebral postnatal MRI performed at 1 month of age confirmed the hypoplasia of the falx cerebri (absent in its anterior part), agenesis of the corpus callosum, and septum pellucidum. It also allowed the visualization of gyration anomalies, focal polymicrogyria as well as subependymal heterotopias of gray matter at the left ventricular crossroads. At 3 months, the patient's follow-up was reassuring in terms of her neurological evolution. At 9 months, the girl was hypotonic and a neurological physiotherapy support was set up once a week. Then, at 11 months the neurological evolution was favorable.

3 | GENETIC ANALYSIS

The parents gave written consents for them and the index case for the participation in this study (approved by local Hôpital Erasme Ethical committee under P2016/236 reference). Array-CGH was performed on a CytoSure™ Constitutional v3 8x60K

FIGURE 1 A, Midsagittal slice showing the agenesis of the posterior part of the corpus callosum, the fused parenchyma on the midline (arrow), and the retrocerebellar arachnoid cyst (arrow head). B, Coronal slice through the third ventricle showing the parenchymal bridge (arrow). C, D, Axial slices showing the cerebellar hypoplasia (C) and the parenchymal bridge (D, arrow)



array (Oxford Gene Technology) and analyzed with CytoSure Analysis Software (Oxford Gene Technology). Clinical exome sequencing was performed in duo (on fetal DNA extracted directly from amniotic fluid and maternal DNA [paternal DNA was not available at that time]), using an in-house SeqCap EZ choice XL capture (Roche Nimblegen) on a NovaSeq 6000 (Illumina) at the Brussels Interuniversity Genomics High Throughput core (BRIGHTcore). Sequences were aligned to the reference genome (hg19) and variants were called using a BWA-mem Unified Genotyper-Haplotype Caller GATK pipeline. Filtering of the variants was accomplished using Highlander (<http://sites.uclouvain.be/highlander>). Details on pipeline and filtering are available on request. Sanger sequencing was performed for the validation of the detected *ZIC2* variant. DNA was amplified using a standard PCR (primers sequences are available upon request). PCR products were purified with BigDye® X Terminator™ Purification Kit (Applied Biosystems, Thermo Fischer Scientific) and analyzed on a 3130XL Genetic Analyser (Biosystems).

4 | DISCUSSION

Here, we described the first MIHV case displaying a *ZIC2* missense mutation (NM_007129.5: c.1109G>A p.(Cys370Tyr)). Our detected variant is not present in

frequency databases (gnomAD, Exome Variant Server) and several prediction tools (SIFT, MutationTaster, FATHMM, PolyPhen-2, DEOGEN¹⁴) agree to predict this change as deleterious. Like a considerable part of all *ZIC2* mutations,¹⁵ our variant is localized into the well conserved zinc finger domain of the protein. To date, only five variants (one splice variant, one small deletion, and three small duplications) in the *ZIC2* gene have been described in MIHV (Figure 2C).^{16,17} As a matter of fact, the majority of *ZIC2* mutations generally result in classic HPE, and more often in severe structural brain anomalies (alobar or semilobar presentations) which account for 75% of the *ZIC2*-associated HPE cases in which the phenotype is recorded.¹⁵ Moreover, our variant arose as a de novo event in the fetus, similarly to the data provided by several HPE cohorts showing that *ZIC2* mutations appear often de novo, with inherited mutations accounting for 27%–30%.^{9,11,17} Focusing on the *ZIC2*-associated MIHV, *ZIC2* mutations appeared de novo in three cases, while in the other two the mutation was inherited (in one case from the father and in the other from the mother, with an assumed germline mosaicism).¹⁷

To date, our case represents the first prenatal report of *ZIC2*-associated MIHV. Overall, only six prenatal reports of MIHV, including 10 fetuses, are described in the literature. The presence of chromosomal anomalies solved six cases (monosomy 13q and 21q were found in five and one

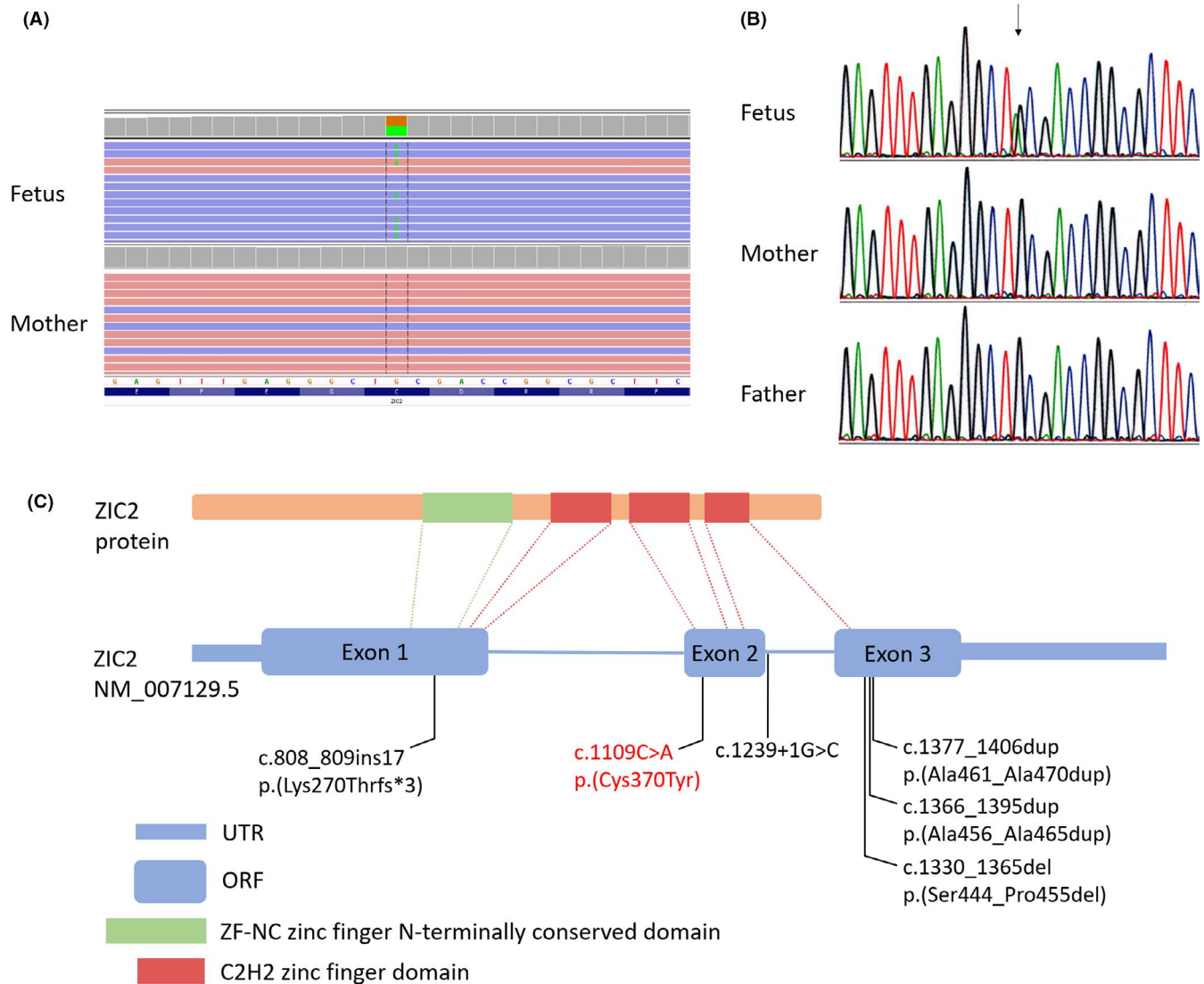


FIGURE 2 A, De novo c.1109G>A p.(Cys370Tyr) variant in exon 2 of the ZIC2 gene visualized in IGV. B, Presence of the mutation in the fetus but none of the parent was confirmed by Sanger sequencing. C, Schematic representation of the ZIC2 gene and its protein (with the main protein domains) along with the mutation described in MIHV until now (data collected from Ref.14,15). Our missense mutation is shown in red

case, respectively) and a normal karyotype was observed in the remaining four cases and no other genetic tests were performed. Among these 10 prenatal cases, medical termination of pregnancy has been carried out in seven cases.¹⁸⁻²⁰ Pulitzer et al²¹ described a fetus carrying several extracerebral malformations who died in utero. Robin et al²² reported a fetus presenting an isolated syntelencephaly who was born at term and with a normal neonatal development at 2 months. For the last prenatal case, the antenatal and postnatal evolutions were not transmitted. Overall, our patient represents the eleventh case of antenatal MIHV diagnosis and the second case born alive. In particular, our case displayed the classic radiological signs of MIHV and its associated abnormalities, including posterior fossa malformation (cerebellar hypoplasia and arachnoid cyst), subependymal heterotopias, and tight areas of polymicrogyria.

Nineteen percent of MIHV patients described in Simon's report (21 children) have a malformation of the posterior fossa, including one case of cerebellar hypoplasia.²³ Interestingly, no arachnoid cysts have previously been described in the literature. The co-occurrence of MIHV and cerebellar malformations in patients with ZIC2 mutations may be explained by its involvement in the neural tube closure. Furthermore, malformations of the posterior fossa (like Chiari [types 1 and 2] and cephalocele) are also often being associated with MIHV.²³ Foci of subependymal heterotopia and polymicrogyria of gray matter appear to be relatively common in MIHV cases. They have been described in six postnatal case studies and 86% of the children in Simon's study had cortical dysplasia.²³ Regarding the hypothalamic-pituitary axis, it appeared intact in our report as well as in the six antenatal studies, whereas it

has been described as hypoplastic in two postnatal studies.²⁴ In Simon's cohort, 23% of the children had pituitary gland hypoplasia. Endocrine disorders are therefore also less frequent in MIHV than in holoprosencephaly, where midline abnormalities frequently initiate the development of the hypothalamus and pituitary gland.¹ Associated extracerebral abnormalities, including two types of cardiac abnormalities (transposition of the great vessels, situs inversus with interventricular septum anomaly), have been described in seven fetuses and two children (under 1 year of age).^{25,26} One patient showed diaphragmatic hernia with vertebral malformations²¹ and another presented cleft palate, clubfoot, and hypospadias.²⁷ In some reports, five fetuses and 15 children displayed facial dysmorphism such as cleft lip and palate and hypertelorism, limb abnormalities, external genitalia, and renal hypoplasia.^{18,28} In these cases, the genetic studies (karyotype) were normal, with the exception of five cases where a 13q monosomy was found.¹⁸

Moreover, craniofacial anomalies are common in HPE but the majority of MIHV patients present moderate facial dysmorphism or even a normal face,^{18,28} like in our case. The different embryological origin of MIHV and HPE explains this clinical difference: MIHV arises as a consequence of failed dorsal patterning, while HPE is associated with failed ventral patterning. Thus, *ZIC2* gene is believed to be responsible for the more posterior defects presented in MIHV, being less often associated with facial malformations. Interestingly, it has been shown that even severe HPE linked to *ZIC2* genes have mild facial dysmorphic features. However, a typical facial phenotype can be associated in *ZIC2*-associated HPE, including bitemporal narrowing, up slanting palpebral fissures, flat nasal bridge, short nose with anteverted nares, broad and deep philtrum, and large ears.¹⁷

Regarding the postnatal clinical outcome of our case, the patient has no dysmorphism and she seems to present subnormal neurological development at 11 months. Previous studies identified spasticity as the main clinical sign of MIHV (found in 86% of children in Lewis's study²⁸), probably due to the fact that the abnormal fusion zone of the cortex is close to the motor cortex. In addition, 57% of patients display some degree of hypotonia, overlapping with our patient who needed physiotherapy support for hypotonia at 9 months. Although choreoathetosis has been frequently found in semilobar holoprosencephaly, no cases displaying this symptom have been described in MIHV.²⁸ This is probably due to the fact that, conversely to HPE, caudate and lenticular nuclei are normal in MIHV.

In conclusion, our findings expand the mutational spectrum and delineate the prenatal phenotype found in *ZIC2*-associated MIHV. As there are limited prenatal MIHV cases harboring *ZIC2* mutations, this work may be an important support for the prenatal diagnosis and the subsequent management of MIHV cases.

ACKNOWLEDGMENTS

The authors thank the family who gave its written consent for this publication. The authors acknowledge all the members of the Molecular Genetics laboratory of Hôpital Erasme for their valuable technical assistance. They also thank all the members of the Saint-Pierre UHC fetal medicine team for their participation in the management of this clinical case. The authors acknowledge Guillaume Smits for critically reading the manuscript. MM is supported by a FNRS-FRIA fellowship.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

CG and MM: wrote the article. VG: examined the patient. MD and MM: analyzed the genetics data. PJ: served as the author of the postnatal MRI. MC: served as the author of the antenatal MRI. MC and JD: coordinated and approved the final version of the manuscript.

ORCID

Caroline Gounongbé  <https://orcid.org/0000-0002-7408-883X>

Martina Marangoni  <https://orcid.org/0000-0002-9416-2400>

Marie Cassart  <https://orcid.org/0000-0003-2766-1805>

REFERENCES

- Dubourg C, Bendavid C, Pasquier L, Henry C, Odent S, David V. Holoprosencephaly. *Orphanet J Rare Dis*. 2007;2:8.
- Marcorelles P, Laquerriere A. Neuropathology of holoprosencephaly. *Am J Med Genet C Semin Med Genet*. 2010;154C(1):109-119.
- Hahn JS, Barnes PD, Clegg NJ, Stashinko EE. Septopreoptic holoprosencephaly: a mild subtype associated with midline craniofacial anomalies. *AJNR Am J Neuroradiol*. 2010;31(9):1596-1601.
- Barkovich AJ, Quint DJ. Middle interhemispheric fusion: an unusual variant of holoprosencephaly. *AJNR Am J Neuroradiol*. 1993;14(2):431-440.
- Bendavid C, Dupé V, Rochard L, Gicquel I, Dubourg C, David V. Holoprosencephaly: an update on cytogenetic abnormalities. *Am J Med Genet C Semin Med Genet*. 2010;154C(1):86-92.
- Pineda-Alvarez DE, Dubourg C, David V, Roessler E, Muenke M. Current recommendations for the molecular evaluation of newly diagnosed holoprosencephaly patients. *Am J Med Genet C Semin Med Genet*. 2010;154C(1):93-101.
- Roessler E, Muenke M. The molecular genetics of holoprosencephaly. *Am J Med Genet C Semin Med Genet*. 2010;154C(1):52-61.
- Barr M, Cohen MM. Autosomal recessive alobar holoprosencephaly with essentially normal faces. *Am J Med Genet Part A*. 2002;112(1):28-30.
- Mercier S, Dubourg C, Garcelon N, et al. New findings for phenotype-genotype correlations in a large European series of holoprosencephaly cases. *J Med Genet*. 2011;48(11):752-760.
- Ming JE, Kaupas ME, Roessler M, et al. Mutations in *PATCHED-1*, the receptor for *SONIC HEDGEHOG*, are associated with holoprosencephaly. *Hum Genet*. 2002;110(4):297-301.

11. Mouden C, Dubourg C, Carré W, et al. Complex mode of inheritance in holoprosencephaly revealed by whole exome sequencing. *Clin Genet*. 2016;89(6):659-668.
12. Roessler E, Vélez JI, Zhou N, Muenke M. Utilizing prospective sequence analysis of SHH, ZIC2, SIX3 and TGIF in holoprosencephaly probands to describe the parameters limiting the observed frequency of mutant gene×gene interactions. *Mol Genet Metab*. 2012;105(4):658-664.
13. Roessler E, Lacbawan F, Dubourg C, et al. The Full Spectrum of Holoprosencephaly-Associated Mutations within the ZIC2 Gene in Humans Predicts Loss-of-Function as the Predominant Disease Mechanism. *Hum Mutat*. 2009;30(4):E541-E554.
14. Raimondi D, Gazzo AM, Rooman M, Lenaerts T, Vranken WF. Multilevel biological characterization of exomic variants at the protein level significantly improves the identification of their deleterious effects. *Bioinformatics*. 2016;32(12):1797-1804.
15. Barratt KS, Arkell RM. ZIC2 in holoprosencephaly. *Adv Exp Med Biol*. 2018;1046:269-299.
16. Nakayama J, Kinugasa H, Ohto T, et al. Monozygotic twins with de novo ZIC2 gene mutations discordant for the type of holoprosencephaly. *Neurology*. 2016;86(15):1456-1458.
17. Solomon BD, Lacbawan F, Mercier S, et al. Mutations in ZIC2 in human holoprosencephaly: description of a novel ZIC2 specific phenotype and comprehensive analysis of 157 individuals. *J Med Genet*. 2010;47(8):513-524.
18. Marcorelles P, Loget P, Fallet-Bianco C, Roume J, Encha-Razavi F, Delezoide A-L. Unusual variant of holoprosencephaly in monosomy 13q. *Pediatr Dev Pathol*. 2002;5(2):170-178.
19. Tran Mau-Them A, Goumy C, Delabaere A, Laurichesse-Delmas H, Lemery D, Gallot D. [Middle interhemispheric variant of holoprosencephaly and partial 21q monosomy]. *Gynecol Obstet Fertil*. 2015;43(4):326-327.
20. Vasudeva A, Nayak SS, Kadavigere R, Girisha KM, Shetty J. Middle interhemispheric variant of holoprosencephaly - presenting as non-visualized cavum septum pellucidum and an interhemispheric cyst in a 19-weeks fetus. *J Clin Diagn Res*. 2015;9(9):QD11-QD13.
21. Pulitzer SB, Simon EM, Crombleholme TM, Golden JA. Prenatal MR findings of the middle interhemispheric variant of holoprosencephaly. *AJNR Am J Neuroradiol*. 2004;25:1034-1036.
22. Robin NH, Ko LM, Heeger S, Muise KL, Judge N, Bangert BA. Syntelencephaly in an infant of a diabetic mother. *Am J Med Genet Part A*. 1996;66(4):433-437.
23. Simon EM, Hevner RF, Pinter JD, et al. The middle interhemispheric variant of holoprosencephaly. *AJNR Am J Neuroradiol*. 2002;23(1):151-156.
24. Bulakbasi N, Cancuri O, Kocaoğlu M. The middle interhemispheric variant of holoprosencephaly: magnetic resonance and diffusion tensor imaging findings. *Br J Radiol*. 2016;89(1063):20160115.
25. Merrow AC, Shah R. Syntelencephaly: postnatal sonographic detection of a subtle case. *Pediatr Radiol*. 2010;40(Suppl 1):S160.
26. Posada M, Castillo M. Clinical image. Middle interhemispheric variant of holoprosencephaly. *Pediatr Radiol*. 2010;40(11):1843.
27. Takanashi J, Barkovich J, Clegg NJ, Delgado MR. Middle interhemispheric variant of holoprosencephaly associated with diffuse polymicrogyria. *AJNR Am J Neuroradiol*. 2003;24:394-397.
28. Lewis AJ, Simon EM, Barkovich AJ, et al. Middle interhemispheric variant of holoprosencephaly: a distinct cliniconeuro-radiologic subtype. *Neurology*. 2002;59(12):1860-1865.

How to cite this article: Gounongbé C, Marangoni M, Gouder de Beauregard V, et al. Middle interhemispheric variant of holoprosencephaly: First prenatal report of a ZIC2 missense mutation. *Clin Case Rep*. 2020;8:1287–1292. <https://doi.org/10.1002/ccr3.2896>