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Rare metabolic disease mimicking *COL4A1/COL4A2* fetal brain phenotype

T. COSTE^{1,2}, C. ALOUI^{1#}, F. PETIT^{3#}, S. MOUTTON^{4#}, L. DEVISME⁵, C. F. WELLS⁶, N. LÉBOUCQ⁷, P. VERPILLAT⁸, M. YVERT⁴, F. RIVIER^{9*} and E. TOURNIER-LASSERVE^{1,2*}

1 *Université Paris Cité, Inserm, Neuro Diderot, Paris, France;*

2 *Service de Génétique Moléculaire Neurovasculaire, AP-HP, Hôpital Saint-Louis, Paris, France;*

3 *CHU Lille, Clinique de Génétique Guy Fontaine, Lille, France;*

4 *Centre Pluridisciplinaire de Diagnostic Prénatal, MSP Bordeaux Bagatelle, Talence, France;*

5 *CHU Lille, Institut de Pathologie, Lille, France;*

6 *CHU Montpellier, Département de Génétique Médicale et Foetopathologie, Montpellier, France;*

7 *CHU Montpellier, Département de Neuroradiologie, Neuroradiologie Diagnostique Pédiatrique, Montpellier, France;*

8 *CHU Lille, Service de Radiologie, Lille, France;*

9 *CHU Montpellier, Département de Neurologie Pédiatrique, PhyMedExp, Université de Montpellier, INSERM, CNRS, Montpellier, France*

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Correspondence to:

Dr T. Coste, AP-HP, Service de Génétique Moléculaire Neurovasculaire, Hôpital Saint-Louis, Paris, France

(**e-mail:** thibault.coste@aphp.fr)

C.A., F.P. and S.M. contributed equally to this work.

* F.R. and E.T.-L. are co-last authors.

ABSTRACT

Pathogenic variants of collagen type IV alpha 1 and 2 (COL4A1/COL4A2) genes cause various phenotypic anomalies, including intracerebral hemorrhage and a wide spectrum of developmental anomalies.

Only 20% of fetuses referred for COL4A1/COL4A2 molecular screening (fetuses with a suspected intracerebral hemorrhage) carry a pathogenic variant in these genes, raising questions regarding the causative anomaly in the remaining 80% of these fetuses. We examined, following termination of pregnancy or in-utero fetal death, a series of 113 unrelated fetuses referred for COL4A1/COL4A2 molecular screening, in which targeted sequencing was negative. Using exome sequencing data and a gene-based collapsing test, we searched for enrichment of rare qualifying variants in our fetal cohort in comparison to the Genome Aggregation Database (gnomAD) control cohort (n=71 702). Qualifying variants in pyruvate dehydrogenase E1 subunit alpha 1 (PDHA1) were overrepresented in our cohort, reaching genome-wide significance ($P=2.11\times 10^{-7}$). Heterozygous PDHA1 loss-of-function variants were identified in three female fetuses. Among these three cases, we observed microcephaly, ventriculomegaly, germinolytic pseudocysts, agenesis/dysgenesis of the corpus callosum and white-matter anomalies that initially suggested cerebral hypoxic-ischemic and hemorrhagic lesions. However, a careful a-posteriori reanalysis of imaging and postmortem data showed that the observed lesions were also consistent with those observed in fetuses carrying PDHA1 pathogenic variants, strongly suggesting that these two phenotypes may overlap. Exome sequencing should therefore be performed in fetuses referred for COL4A1/COL4A2 molecular screening which are screen-negative, with particular attention paid to the PDHA1 gene.

CASE SERIES

Exome sequencing was performed in 113 fetuses referred initially (with a suspected intracerebral hemorrhage) for collagen type IV alpha 1 and 2 (COL4A1/COL4A2) gene screening, in which no pathogenic variation was identified. A gene-based collapsing burden test comparing qualifying variants in the 113 fetuses vs the Genome Aggregation Database (gnomAD) public control database ranked the pyruvate dehydrogenase E1 subunit alpha 1 (PDHA1) gene first, showing genome-wide significance ($P=2.11\times 10^{-7}$). (For further details of methods, see Appendix S1.) Three qualifying variants of PDHA1 were identified, one in each of three heterozygous female fetuses (Table S1, Figure 1). Two were loss-of-function variants and one was a missense variant. None of these variants was present in public control databases of polymorphisms (gnomAD and the 1000Genomes Project Phase 3 database). The p.(Arg304*) and p.(Arg119Trp) variants have been reported previously, in a large series of patients with pyruvate dehydrogenase complex deficiency (PDCD) (MIM# 312170)¹. The main ultrasound findings and careful a-posteriori reanalysis of magnetic resonance imaging (MRI) features of the affected fetuses are summarized in Table 1. This study was approved by the INSERM ethics review committee (IRB00003888) and informed consent was obtained from parents and for the fetuses included in the study.

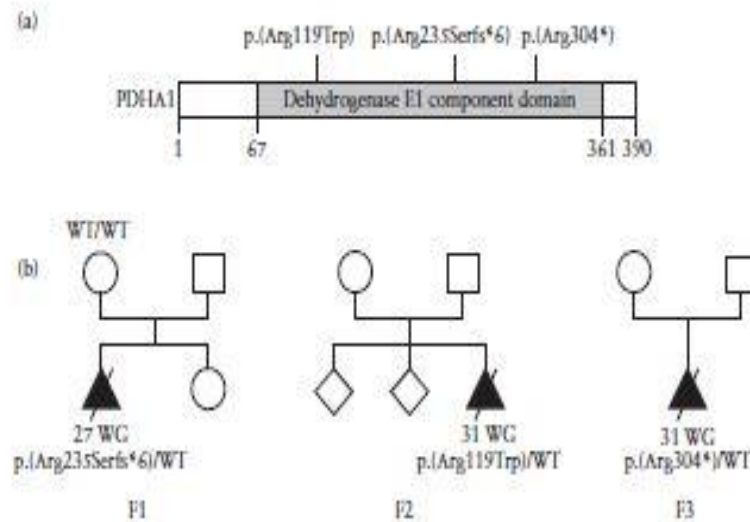


Figure 1 Location of pathogenic *PDHA1* variants and pedigrees of the three fetuses with qualifying mutations. (a) Protein structure and corresponding domain organization of human *PDHA1* protein (protein NP_000275.1). Numbers below the figure represent the first and last coding amino acids for the only protein domain of *PDHA1*, and the last coding amino acid (AA 390). (b) Pedigrees of the three mutated probands. Males are represented by squares and females by circles, triangles indicate pregnancy not carried to term and diamonds indicate gender not specified. Filled symbols indicate affected individual (confirmed clinically and by magnetic resonance imaging) and empty symbols indicate clinically healthy individual. Diagonal black line indicates deceased individual. WC, gestational weeks; WT, wild type.

Table 1 Summary of main clinical and imaging findings in three fetuses with a *PDHA1* variant

Case	GA (weeks)	Ultrasound findings	Brain MRI findings	Brain autopsy findings	Pathogenic variant in <i>PDHA1</i> (NM_000284.4)
F1	21	Microcephaly, corpus callosal agenesis, cerebellar hypoplasia, dilated frontal horns and thickened ventricular walls	Agenesis of posterior part of corpus callosum, agyria, periventricular germinolytic pseudocysts, cerebellar hypoplasia, leukomalacia	Microcephaly, delayed gyration, partial corpus callosal agenesis, subependymal germinolytic pseudocysts, occipital leukomalacia, negative Perls staining†	c.705_706del; p.(Arg235Serfs*6)
F2	24	Ventriculomegaly	Ventriculomegaly, periventricular germinolytic pseudocysts, thin corpus callosum, white-matter abnormalities	Microcephaly, ventriculomegaly, delayed gyration, subependymal germinolytic pseudocysts, thin corpus callosum, negative Perls staining†	c.355C>T; p.(Arg119Trp)
F3	23	Ventriculomegaly, corpus callosal agenesis, echogenic ribbon circumscribing ventricular walls	Microcephaly, cerebellar hypoplasia, ventriculomegaly, almost complete corpus callosal agenesis, parenchymal rarefaction with periventricular germinolytic pseudocysts, delayed gyration	Not performed	c.910C>T; p.(Arg304*)

†Negative Perls staining indicates that there was no sign of previous hemorrhage. GA, gestational age at first suspicious ultrasound examination; MRI, magnetic resonance imaging.

Case 1

Fetus F1 was the first child of unrelated and healthy parents, without familial history. The first trimester of pregnancy was unremarkable. Ultrasound examination suggestive of bilateral ventricular hemorrhage, with hyposignal on T2- and hypersignal on T1-weighted sequences. Given these findings, we concluded that there had been several hypoxic-ischemic and hemorrhagic injuries associated with neurodevelopmental alterations. The fetal blood count and karyotype were normal. The pregnancy was terminated at 27 weeks. Autopsy showed a female fetus with a weight of 908 g, crown–heel length of 36 cm and moderate microcephaly (head circumference, 24 cm). Macroscopic cerebral study revealed global microcephaly with delayed gyration, partial corpus callosal agenesis and parietal and frontal subependymal pseudocysts. Histological analysis showed occipital leukomalacia, fragmentation of olivary and dentate nuclei and agenesis of the pyramidal tracts. Hemosiderin (a sign of previous hemorrhage) was not detected by Perls staining (Figure 3d). These findings were suggestive of clastic events.

Case 2

Fetus F2 was a female fetus from the third pregnancy of a 34-year-old woman. Her two previous children had normal neurological development. Ultrasound examination at 24 gestational weeks found dilatation of the lateral ventricles and a suspected blood clot in the left lateral ventricle without any other abnormality. Fetal brain MRI at 26 weeks confirmed slight asymmetric ventricular dilatation (width of cerebral right lateral ventricle, 14 mm; left lateral ventricle, 12.5 mm) and revealed the presence of three microcysts, which were interpreted as germinolytic cysts, in the left lateral ventricle. A possible left parietal parenchymal ischemic lesion was also suspected. Fetal MRI performed at 30 weeks showed no change in the dilatation of the lateral ventricles. A subependymal hemorrhage was suspected, without any clot in the ventricles. There were germinolytic microcysts in the right lateral ventricle apparently identical to those in the left one, localized in the ganglionic eminence and organized in a ‘string-of-beads’ formation. Microcysts were also present in the anterior part of the lateral ventricles with a septate appearance. The corpus callosum appeared thin. An abnormal signal was noted in the deep and superficial white matter of the left parietal hemisphere, on both T2 and diffusion sequences (Figure 2g–i). Cerebral maturation delay was also observed. Biometric data were between the 25th and 50th percentiles. According to the imaging findings, we concluded that the ventriculomegaly was probably the consequence of an intraventricular hemorrhage associated with ischemic lesions in the white matter and a delay in cerebral maturation. After termination of pregnancy, fetal postmortem examination at 31 weeks showed a eutrophic female fetus with a brain weight below the 5th percentile. Macroscopic brain analysis revealed delayed gyration and moderate dilatation of the lateral ventricles, with subependymal cysts at the ventricular junction and other confluent cysts in the right frontal pole (Figure 3e). The corpus callosum appeared thin without agenesis. Perls staining was negative, including around the different cysts.

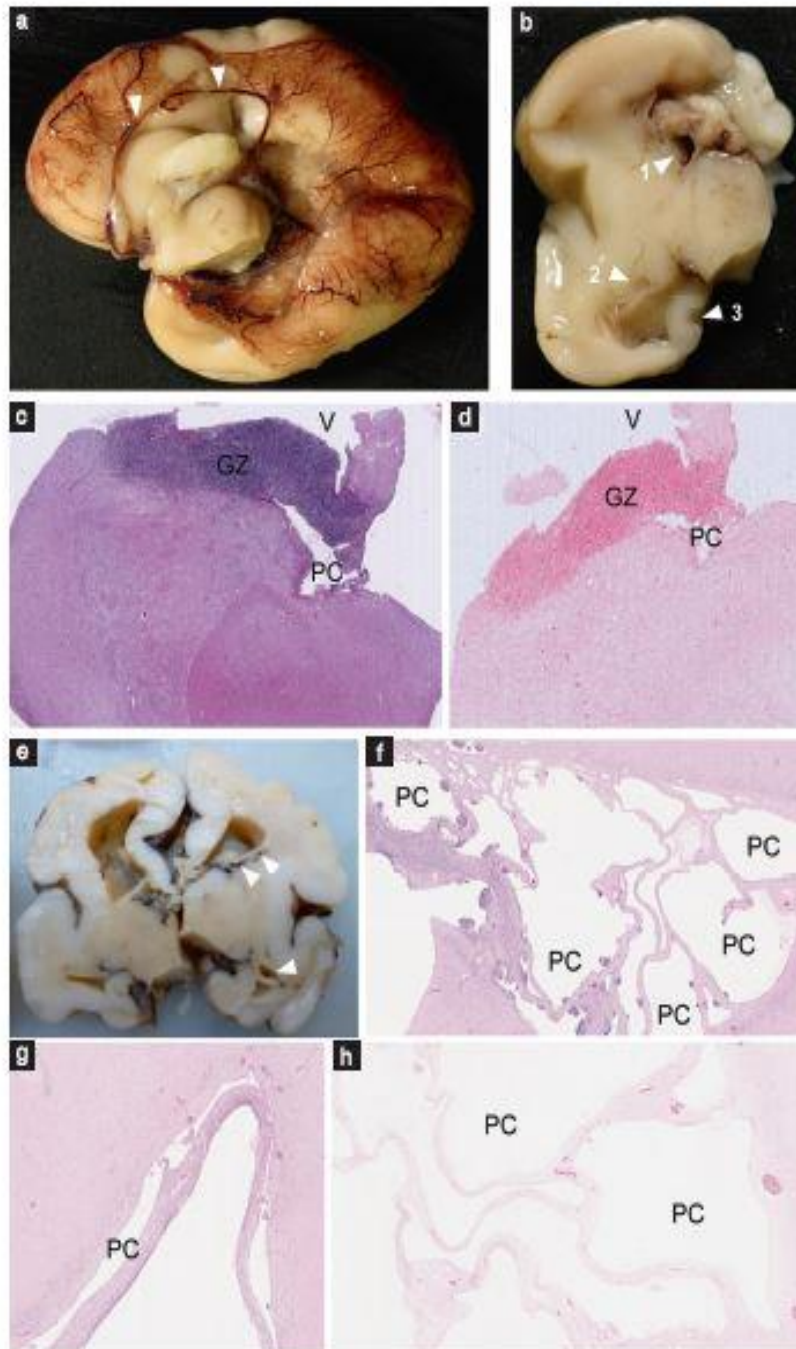


Figure 3 Macroscopic and histological brain features of fetal PDHA1 deficiency. (a–d) Fetus F1, at 27 weeks. (a) Median sagittal slice of right hemisphere showing partial agenesis of the corpus callosum (only third anterior part); arrowheads indicate pericallosal artery. (b) Transverse section of cerebral hemisphere showing left subependymal parietal (arrowhead 1) and temporal (arrowhead 2) pseudocysts (PCs) and temporal thinning (arrowhead 3). (c) Histological image showing subependymal PCs (hematoxylin & eosin staining (HES); original magnification, $\times 25$). (d) Corresponding Perls staining, showing negative result (original magnification, $\times 25$). (e–h) Fetus 2, at 31 weeks. (e) Coronal brain slice showing moderate ventricular dilatation and periventricular PCs (arrowheads). (f) Histological pattern showing multiple frontal PCs (HES; original magnification, $\times 20$). (g) Periventricular subependymal PC (HES; original magnification, $\times 20$). (h) Frontal PCs with negative result for Perls staining (original magnification, $\times 40$). GZ, germinative zone; V, ventricle.

Case 3

Fetus F3 was from the second pregnancy of an unrelated couple who already had a healthy child, without any familial history. Ultrasound examination at 23 gestational weeks found apparently complete corpus callosal agenesis with mild ventricular enlargement. Karyotyping

and polymerase chain reaction analysis for cytomegalovirus were negative. A second ultrasound examination at 28 weeks confirmed the corpus callosal agenesis and dilatation of the lateral ventricles (Figure 2n). On brain MRI at 28 weeks (Figure 2j–m), the head circumference was below the 3rd percentile. The cerebral parenchyma showed overall rarefaction, with widened pericerebral spaces. The corpus callosum was almost entirely absent, although a small central segment could be observed (8mm in length from the anteroposterior axis and 2mm in width). The septal cavity was absent. The third ventricle was dilated and appeared ascended. Bilateral ventriculomegaly was slightly asymmetrical (left lateral ventricle, 15 mm; right lateral ventricle, 13 mm). The frontal ventricular horns were dilated and the left frontal ventricular horn presented relatively rectangular contours. The ventricular walls were irregular and on the left side they were relatively indented, without the appearance of nodular heterotopia. Several pseudocystic zones suggestive of germinolytic lesions were observed, located particularly within the left basifrontal white matter and to a lesser extent within the right frontal subependymal region, and bilaterally within the germinative zone in the periphery of the temporal horns. Sagittal sequences showed a slit-like appearance of the lateral ventricular walls, suggesting extensive germinolytic lesions, probably corresponding to the echogenic ribbon that surrounds the ventricular wall on ultrasound. Additionally, there was a clear indentation of the left anterior frontal cortex and adjacent white matter, located at the level of the interhemispheric fissure paramedially, with a lacunar appearance, without radiological evidence of schizencephaly. The T1- and T2-weighted gradient echo sequences were not suggestive of hemorrhagic stigma. Although it was still relatively early in gestation, a clear and global delay in gyration was noted. At the subtentorial level, the cerebellar growth parameters were below the 3rd percentile. The vermis appeared complete and the morphology of the fourth ventricle was normal. There was significant widening of all pericerebellar fluid spaces. Overall, a very early clastic event was hypothesized. The pregnancy was terminated at 31 weeks and the parents declined postmortem examination.

DISCUSSION

Our findings highlight that *PDHA1* pathogenic variants may lead to ultrasound and brain MRI lesions suggestive of those observed in fetuses carrying *COL4A1/COL4A2* pathogenic variants. In addition, our data strongly suggest the utility of exome sequencing in fetuses referred for *COL4A1/COL4A2* screening for which targeted sequencing is negative.

Fetal brain anomalies of *PDHA1* deficiency could lead to suspicion of lesions associated with *COL4A1/COL4A2* pathogenic variants

Primary PDCD is a mitochondrial disorder of carbohydrate oxidation. Molecular alterations in the *PDHA1* gene are the main cause of PDCD with an X-linked dominant inheritance pattern and a high frequency of *de-novo* variants (85–95% in female and 60–63% in male cases)². The clinical phenotype and phenotype on imaging as well as the age at onset of PDCD are variable, the median age at onset being about 20 months^{3,4}. PDCD may also present during fetal life, with prenatal ultrasound or MRI detecting microcephaly, ventriculomegaly, paraventricular pseudocysts, cerebellar hypoplasia, delayed gyration and/or dysgenesis of the corpus callosum. In addition, stroke-like lesions have been reported in some fetuses⁵. Fetal autopsy features have been described by Pirot *et al.*⁶ in two unrelated female fetuses with *PDHA1* variants. The first patient had partial agenesis of the corpus callosum and microcephaly on routine ultrasound at 22 gestational weeks. Fetal MRI showed agenesis of

the corpus callosum, microcephaly, colpocephaly, delayed gyral formation, cerebellar hypoplasia and microcavitary periventricular white-matter lesions. Macroscopic and histologic examination of the brain showed pachygyria, polymicrogyria, ventricular dilatation, pseudocysts of the subependymal germinal matrix and basal ganglia microcalcifications. The second fetus had agenesis of the corpus callosum, mild ventricular dilatation and delayed gyration at the 22-week ultrasound examination. Postmortem examination showed extensive subcortical cavitory lesions in the brain, corpus callosal hypoplasia, pseudocysts of the subependymal germinal matrix, dilatation of the ventricles, reactive gliosis and ischemic lesions.

Several clinical and imaging manifestations encountered in PDHA1 deficiency are also encountered in *COL4A1/COL4A2* collagenopathy. First, microcephaly can be observed in both conditions, although less consistently in *COL4A1/COL4A2* collagenopathy⁷. Second, ventriculomegaly is a common element in both cases^{6,8}, although it is often classified as moderate in metabolic abnormalities compared with those associated with a hemorrhagic event. Hypoplasia of the corpus callosum, cerebellar hypoplasia, schizencephaly, gyration abnormalities and periventricular leukomalacia are also elements that can be observed in both PDHA1 deficiency and *COL4A1/COL4A2*-related disorders^{7,9,10}. Dysgenesis of the corpus callosum in *PDHA1* fetuses is thought to be developmental; this is in contrast to the corpus callosal lesions of *COL4A1* fetuses, which are thought to be the consequence of clastic lesions⁶. For this reason, an early anomaly of the corpus callosum should lead to suspicion of a *PDHA1* mutation. Finally, intracranial calcifications may be observed in both *COL4A1/COL4A2* collagenopathy and PDHA1 deficiency⁶. Some elements, however, tend to be specific to only one of the two conditions. To our knowledge, periventricular germinolytic pseudocysts have not been reported in patients with pathogenic variants in *COL4A1/COL4A2*. Hemorrhages are usually not detected on brain histological examination, even after Perls staining, in fetuses with PDHA1 deficiency, in contrast to observations in most *COL4A1/COL4A2* fetuses. Altogether, these data emphasize the overlap of *PDHA1* and *COL4A1/COL4A2* phenotypic features in fetuses carrying pathogenic variants.

Shared phenotypic features of fetuses in this series

In our three cases, antenatal brain imaging using ultrasound and particularly MRI raised the possibility of hypoxic-ischemic and/or hemorrhagic lesions, without demonstrating them clearly (non-specific white-matter abnormalities; paraventricular microcysts interpreted as germinolysis without certainty; absence of signs of intraventricular or intraparenchymal hemorrhage). In fetuses F1 and F3, the first sign of concern was an abnormal or absent corpus callosum (partial agenesis of the posterior part in F1 and almost complete agenesis in F3). In fetus F2, the corpus callosum appeared normal on ultrasound examination but a thin corpus callosum was then observed on fetal brain MRI. These corpus callosal abnormalities were confirmed by postmortem examination in two cases; postmortem was not carried out in fetus F3. All three fetuses presented the appearance of pseudo- and/or microcysts at the subependymal level, suggesting germinolysis. In F2, they appeared localized in the ganglionic eminence and organized in a 'string-of-beads' formation. In the same fetus, there were also microcysts in the anterior part of the lateral ventricles with a septate appearance, which was not suggestive of germinolysis. In fetuses F1 and F3, cerebellar hypoplasia was also observed. These different findings are rather unusual for lesions of vascular origin, such as those found in *COL4A1/COL4A2* collagenopathy. When hypoxic-ischemic/hemorrhagic lesions are suspected, it is crucial to examine carefully the appearance of microcysts that are not truly

undergoing germinolysis, and to look for developmental abnormalities of the corpus callosum and cerebellum that may indicate PDHA1 deficiency, particularly in a female fetus.

Exome sequencing: efficient strategy for fetuses with suspected cerebral hypoxic-ischemic lesions and ventriculomegaly

This study emphasizes the difficulty in some cases in achieving accurate diagnosis based on fetal phenotypic analysis. In these three fetuses, imaging findings initially suggested a diagnosis based on a hypoxic-ischemic or hemorrhagic event, which led us to conduct first-line targeted sequencing of the *COL4A1/COL4A2* genes. The final diagnosis was achieved only after an exome-wide analysis performed using a proband-only strategy. Taken together, these data highlight the importance of genome-wide screening after targeted *COL4A1/COL4A2* gene screening, coupled with sequential *in-silico* analysis of candidate genes, based on a multidisciplinary approach.

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