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## **COL4A1/COL4A2 and inherited platelet disorder gene variants in fetuses showing intracranial hemorrhage**

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### **Abstract**

**Background:** Variants of *COL4A1/COL4A2* genes have been reported in fetal intracranial hemorrhage (ICH) cases but their prevalence and characteristics have not been established in a large series of fetuses. Fetal neonatal alloimmune thrombocytopenia is a major acquired ICH factor but the prevalence and characteristics of inherited platelet disorder (IPD) gene variants leading to thrombocytopenia are unknown. Herein, we screened *COL4A1/COL4A2* and IPD genes in a large series of ICH fetuses.

**Methods:** A cohort of 194 consecutive ICH fetuses were first screened for *COL4A1/COL4A2* variants. We manually curated a list of 64 genes involved in IPD and investigated them in *COL4A1/COL4A2* negative fetuses, using exome sequencing data from 101 of these fetuses.

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**CONFLICT OF INTEREST**

Nothing to report.

**ETHICS STATEMENT**

This study was approved by the INSERM Ethics Review Committee (IRB00003888) and an informed and signed consent was obtained for parents and fetus probands included in the study.

**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher's website.

**Result:** Pathogenic variants of *COL4A1/COL4A2* genes were identified in 36 fetuses (19%). They occurred de novo in 70% of the 32 fetuses for whom parental DNA was available. Pathogenic variants in two megakaryopoiesis genes (*MPL* and *MECOM* genes) were identified in two families with recurrent and severe fetal ICH, with variable extraneurological pathological features.

**Conclusion:** Our study emphasizes the genetic heterogeneity of fetal ICH and the need to screen both *COL4A1/COL4A2* and IPD genes in the etiological investigation of fetal ICH to allow proper genetic counseling.

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## 1 | INTRODUCTION

Fetal intracranial hemorrhage (ICH) refers to bleeding that occurs antenatally from a blood vessel into the ventricles, subdural space or brain parenchyma. Whereas neonatal hemorrhage is a relatively common occurrence in preterm infants delivered before 32 weeks of gestation (WG), fetal ICH has an unclear incidence, although an estimate of 1 in 10,000 pregnancies has been suggested.<sup>1</sup> Prenatal diagnosis of fetal ICH is generally made during the second or third trimester by either ultrasound (US) or magnetic resonance imaging (MRI). Fetal ICH has been classified in five types according to the anatomic location of the bleeding: intraventricular (IVH), subarachnoid, intraparenchymal, cerebellar and subdural hemorrhage. IVH are further subdivided in four grades for which extensive hemorrhages are classified as grade III (hemorrhage with ventriculomegaly) and IV (hemorrhage within the cerebral parenchyma).<sup>2</sup> Both are associated with a very poor perinatal outcome including intrauterine fetal death (IUID), or severe neurodevelopmental anomalies that may lead to a termination of pregnancy (TOP) decision.<sup>3</sup>

Fetal ICH can be associated with several acquired maternal and fetal factors including maternal trauma, infection, drug use and alloimmune thrombocytopenia. Among those acquired causes, fetal and neonatal alloimmune thrombocytopenia (fetal neonatal alloimmune thrombocytopenia [FNAIT]) is a well-established etiology of profound thrombocytopenia in fetuses or neonates and is responsible of severe bleedings especially in the brain<sup>4</sup>. On the other hand, variants of *COL4A1* and *COL4A2* genes, which encode basement-membrane proteins, have been reported in several fetal ICH case reports since 2011.<sup>5</sup> A recent meta-analysis conducted on PubMed and EMBASE databases ranging from 1980 to 2020 identified a total of 20 mutated fetal cases.<sup>6</sup> A few additional genes have been shown to be mutated in very rare ICH fetal cases including clotting factors V and VII, protein C and VWF.<sup>6</sup> In a recent study on fetal or neonatal ICH including 9 cases with a prenatal diagnosis and 17 with a postnatal diagnosis, Hausman-Kedem et al.<sup>7</sup> identified a causative/likely causative variant in 12% of them and emphasize the importance of these findings for genetic counseling. However, in most fetal ICH cases the underlying cause is not identified, precluding any genetic counseling.

In this study, we investigated the prevalence and characteristics of *COL4A1* and *COL4A2* gene variants in a cohort of 194 consecutive fetuses for whom TOP was performed or IUID occurred due to fetal ICH and for whom acquired causes have been excluded. We also generated a list of 64 genes known to be involved in inherited platelet disorder (IPD)

by manual curation of the literature and investigated the presence of pathogenic variants in those genes using whole exome sequencing (ES) data from 101 *COL4A1/COL4A2* negative fetuses.

## 2 | MATERIALS AND METHODS

### 2.1 | Case ascertainment and inclusion

From 2010 to 2020 a series of 194 consecutive fetuses interrupted following either TOP or IUFD were referred for *COL4A1* and *COL4A2* genes screening to our molecular genetics reference center in France (national reference center for inherited cerebrovascular disorders, Saint Louis Hospital). Fetal ICH was in most cases detected at systematic second and third trimester US examination and most cases were confirmed by fetal MRI and/or pathological examination. Systematic review of medical charts was performed in order to exclude fetuses with an identifiable cause or known risk factor for ICH. These included evidence of maternal trauma during pregnancy, cocaine or maternal drug use, maternal or neonatal infections and fetal alloimmune thrombocytopenia. Written informed consent for genetic investigation and research was provided by both parents in accordance with the declaration of Helsinki and the French law. This study has been approved by the Inserm Ethics Committee (INSERM IRB00003888).

Genomic DNA was extracted from post mortem fetal tissue and from peripheral blood leukocytes of both parents and relatives, when available.

### 2.2 | Postmortem examination

Complete postmortem examination was performed with the informed consent of both parents in accordance with the French law and following standardized protocols including X-rays, photographs, macroscopical and histological examination of all viscera. Brains were fixed with formalin for 5–12 weeks. Macroscopic analysis was performed allowing the selection and conditioning of samples (paraffin embedding, 7-micron slicing, hematoxylin and eosin stain [HES]) of brain tissue for histological analysis.

### 2.3 | Sequencing of *COL4A1* and *COL4A2* genes

Sanger sequencing (fetuses referred in 2010–2015) or targeted high throughput sequencing of *COL4A1* and *COL4A2* genes (fetuses referred in 2016–2020) was performed for the 194 fetuses included in this study. Library preparation was performed using the SureSelect QXT<sup>®</sup> capture kit (Agilent technologies) and sequencing was performed on a MiSeq<sup>®</sup> sequencer (Illumina). We used SeqNext software 4.4 and 5.0 versions (JSI Medical Systems) to analyze the data. Variants were confirmed by Sanger sequencing using the BigDye<sup>™</sup> Terminator v3.1 (Applied Biosystems) on an ABI 3130 DNA sequencer with in house primers (available upon request). Glycine missense variants or in-frame deletions in the triple helical domain, loss of function variants and rare de novo variants located in the non-collagenous (NC1) domain were classified as pathogenic variants. The de novo nature of variants was established by microsatellite analysis of both fetus and parental DNA using the AmpFISTR Profiler PCR Amplification Kit (Applied Biosystems).

## 2.4 | Investigation of genes leading to inherited platelet disorders

Several genes have been reported to be associated with an IPD, a heterogeneous group of rare disorders characterized by defects in platelet production and/or function. Some IPD genes encode proteins that play a critical role in platelet production leading to an inherited thrombocytopenia (IT), others may affect cytoskeletal proteins, formation of granules, platelet responses to agonists or interactions with extracellular matrix proteins. We manually curated literature data to identify all genes currently known to be involved in IPD.<sup>8-17</sup> A list of 64 IPD genes was generated (Supporting information S1).

Exome sequencing (ES) being a cost-effective approach to screen a large number of candidate genes, we used this strategy to screen the 64 IPD genes on a series of 101 unrelated fetuses without *COL4A1* or *COL4A2* variants and for whom ES research consents were obtained from parents and for whom DNA with a sufficient quality and quantity was available for ES (Figure 1). Libraries were prepared at the IntegraGen platform (Evry, France) for fetuses and their relatives using the SureSelect Human All Exon V5-UTR (Agilent technologies). Libraries were sequenced on a NovaSeq system (Illumina Inc). Data analysis of single-nucleotide substitutions and small insertion-deletion (indel) variants were performed with an IntegraGen bioinformatic pipeline that included standard tools as BWA and Haplotype Caller GVCF tool (GATK 3.8.1). Ensembl's VEP (Variant Effect Predictor, release VEP 95.1) program was used to process variants for further annotation. We used CANOES for the detection of copy number variation (CNV) on ES data.<sup>18</sup>

After removing variants with low quality score, variants in IPD genes were filtered out based on their frequency. We filtered out variants with a Minor Allele Frequency (MAF)  $< 0.001$  in the gno-mAD v2.1.1 and 1000 Genomes phase 3 databases for genes known to be involved in dominant IPD and with a MAF  $< 0.01$  for genes with recessive inheritance. We restrained our analysis to high impact variants (nonsense, frameshift, and splice site variants) and missense substitutions located in coding regions. Missense candidate variants were scrutinized using the PolyPhen-2 in silico pathogenicity prediction software and we excluded variants predicted to be benign. All variants were finally classified using the consensus guidelines set out by the American College of Medical Genetics and Genomics (ACMG guidelines).<sup>19</sup> We considered as candidate variants only those classified as pathogenic or likely pathogenic. All candidate variants were confirmed by Sanger sequencing.

## 3 | RESULTS

### 3.1 | Identification of *COL4A1*/*COL4A2* pathogenic variants in 19% of fetuses

We identified a pathogenic variant in 36 out of the 194 unrelated fetuses referred for diagnosis. Thirty cases showed a pathogenic variant in *COL4A1* and six cases showed a pathogenic variant in *COL4A2*. A total of 35 distinct variants were identified in these patients (one *COL4A1* variant was shared by two fetuses). The vast majority of the 35 distinct pathogenic variants were located in the triple helical domain, including 27 glycine missense variants and 5 splice site variants. Two variants are located in the NC1 domain of *COL4A1* gene (Figure 2). *COL4A1* variants appeared to be clustered on the c-term half

of the triple helix domain. We compared this distribution with the distribution of variants detected in consecutive adult proband patients referred to our laboratory for a diagnostic of vascular leukoencephalopathy (Supporting information S1). A significant difference in the location of the variants in the triple helix domain was observed (One-sided *t*-test,  $p = 0.0028$ ; Supporting information S1).

For 32 fetuses out of the 36 with a *COL4A1/COL4A2* variant, parental DNA was available and molecular screening of parents' DNA showed that 22 of the 32 (70%) fetuses carried a de novo variant.

### 3.2 | Identification of ICH causative variants in inherited platelet disorder genes

Screening of genes involved in IPD disorders was performed on a series of 101 unrelated fetuses negative for *COL4A1/COL4A2*. Filtering on allele frequency and PolyPhen-2 in silico prediction allowed the identification of 68 candidate variants. We further applied ACMG criteria for each variant and classified 11 variants as pathogenic or likely pathogenic in 10 fetuses (Supporting information S1). Eight fetuses carried a heterozygous variant classified as likely pathogenic or pathogenic in *HPS3*, *HPS5*, *ITGA2B*, *ITGB3*, *LYST* and *VWF* gene. Inherited platelet disorders associated with those genes are mainly reported as autosomal recessive ones. Therefore, in the absence of any additional predicted pathogenic missense variant or CNV on the other allele, we could not formally establish the role of these variants in the ICH of these fetuses.

In the two remaining unrelated fetuses causative pathogenic variants were detected in 2 distinct genes. We identified two compound heterozygous variants in the *MPL* gene (c.305G>C [p.R102P] and c.1609C>T [p.R537W]) in the proband of family F1. This fetus belongs to a family with recurrent fetal ICH. Molecular screening of the second affected fetus detected both variants. Each variant was inherited from one distinct parent and none of the two healthy siblings carried both variants (Figure 3A). *MPL* encodes a transmembrane receptor belonging to the homodimeric class I receptor family, and its ligand is thrombopoietin (TPO). *MPL* plays a central role in the regulation of megakaryopoiesis by regulating platelet production in response to TPO.<sup>20</sup> The R102P variant is located in the EpoR ligand binding domain of *MPL* protein (Figure 3B). The variant R102P impairs cell surface expression of *MPL* protein preventing its interaction with TPO.<sup>21</sup> The R537W variant is located in the intracellular domain and is predicted to impair signal transduction of *MPL*.

Finally, we identified a heterozygous frameshift variant (c.1268del [p.S423Lfs\*6]) leading to a premature stop codon in the *MECOM* gene in family F2. Sanger sequencing of his affected fetus sibling identified the same variant in *MECOM*. This heterozygous stop codon variant is located in exon 8 and expected to lead to a nonsense messenger RNA decay and a haploinsufficiency (Figure 4B). Parents' DNA was not available.

### 3.3 | Clinical and neuropathological features of fetuses with causative variants in megakaryopoiesis genes

Main pathological features of ICH fetuses in families F1 and F2 are summarized in Table 1. The proband (II-2) of family F1 was a male fetus from a second spontaneous

pregnancy of clinically healthy non-consanguineous parents (Figure 3A). His 35 years old mother had a splenectomy in childhood because of a hereditary spherocytosis. The first pregnancy was uneventful, and the mother gave birth to a healthy girl (II-1) although the mother underwent a post-partum hemorrhage. For the second pregnancy, US examination at 27 WG revealed a large bilateral ventriculomegaly consistent with an ICH diagnosis. Investigation by array comparative genomic hybridization analysis was normal but fetal blood cell count showed severe anemia and thrombocytopenia (hemoglobin 3 g/dl; platelets 15 Giga/L). FNAIT was suspected but there were no alloantibodies in the maternal serum. A TOP at 28 WG was performed at parental request. Fetal autopsy did not find any skeletal or visceral malformation. Neuropathological examination showed an asymmetry of the cerebral hemispheres and necrosis lesions in the right parietal region (Figure 3C *top-left*). Histological examination with HES showed suffusions in contact with the thick germinal zone; the edge of the ventricle ependymal layer was partially abraded and replaced by ependymal “pseudo-rosettes” (Figure 3C *top-right*). In association, we observed a diffuse ventricular and subarachnoid chronic hemorrhage, including hemosiderin pigments (Figure 3C *bottom-right*) and siderocalcium salts (Figure 3C *bottom-left*). Moreover, a microscopic right parietal parenchymal necrosis in the white matter and a focal parietal cortical polymicrogyria were noted, revealing inconspicuous ischemic lesions. There was not any sign of systemic hemorrhage. For the third spontaneous pregnancy, first trimester US examination performed at 11 WG revealed an increased nuchal translucency (3 mm). Comparative genomic hybridization analysis was normal. No fetal cell blood count could be performed at this early gestational age. IUFD occurred at 14 WG (II-3). An autopsy was performed, and the fetus presented advanced maceration. The brain was completely lysed; nevertheless, histological examination of the cerebral parenchyma identified a relatively bulky blood clot. Finally, in this family the fourth pregnancy was uneventful (II-4).

The proband of family F2 (III-1) was a male fetus from a first pregnancy of a non-consanguineous healthy couple (Figure 4A). Family history was uneventful apart the presence of a cleft palate in the sister's mother. Systematic US examination at 22 WG was normal except for a suspicion of a cardiac outflow tract anomaly. An US control at 27 WG finally revealed an IUFD. Fetal autopsy did not reveal any skeletal nor developmental abnormality except for a pulmonary atresia with ventricular septal defect and an overriding aorta. Brain examination unveiled a hemorrhagic lesion in the right temporal lobe and showed necrotic and hemorrhagic reorganizations in the left temporal lobe. The second pregnancy was uneventful with the delivery of a healthy child (III-2). A recurrent IUFD occurred at 28 WG during the third pregnancy (III-3). In contrast with observations made for the fetus proband III-1, fetal autopsy revealed skeletal malformations with only 11 pairs of ribs, a rough draft of cervical ribs and a synostosis of radius and ulna. Neuropathological analysis of fetus III-3 of the brain showed a weight within the lower limit of normal, and there was a hematoma filling the left sylvian valley and extending to the frontal pole (Figure 4C). On section, the lateral ventricles were dilated while the left sylvian valley was ripped by a hematoma. The brain parenchyma was punctuated by diffuse and multiple petechiae in the cerebral hemispheres, the brainstem and cerebellum. Histological analysis revealed recent (no sign of resorption) and multifocal bleedings, very often centered by a capillary. There was no associated infectious sign.

## 4 | DISCUSSION

In a cohort of 194 consecutive ICH fetuses interrupted or stillbirth and referred for molecular screening of *COL4A1* and *COL4A2* we identified a pathogenic variant in one of these two genes in 19% of cases. These variants occurred de novo in 70% of these fetuses. In addition, three pathogenic variants of *MPL* and *MECOM* genes were shown to cause recurrent ICH in two distinct families.

### 4.1 | *COL4A1* and *COL4A2* pathogenic variants are the most frequent genetic cause of fetal ICH

Several studies have reported the implication of *COL4A1/COL4A2* variants in fetal ICH with a broad phenotype spectrum including, in addition to ICH, porencephaly, cortical malformations and schizencephaly.<sup>5,22-32</sup> However, most of these studies were case reports of one or very few fetuses. To our knowledge, this is the sole large series of ICH fetuses screened for *COL4A1/COL4A2* genes. These data establish that (i) these two genes account for a large proportion of fetal ICH (ii) 70% of these variants occur de novo. These findings are important for genetic counseling.

The 70% de novo rate observed in this fetal cohort is much higher than in series including post-natal cases, either children or adult patients. Indeed, in their excellent review of their own cases and literature data Meuwissen et al.<sup>29</sup> reported a de novo rate of 40%. In small series limited to fetuses, the reported de novo rate is also very high, around 80%.<sup>5,32</sup> It was also close to 80% in the subgroup of patients with prenatal manifestations in Itai et al recent series.<sup>33</sup> In addition to this high de novo rate, we showed that the distribution of variants along the *COL4A1* gene is significantly different between fetuses and adult patients. Altogether, these data strongly suggest that the very early onset and severity of fetal ICH is at least partly explained by differences in mutations location. Interestingly, this observation is in adequation with mouse models in which severity is also correlated with mutations location.<sup>34,35</sup>

### 4.2 | Thrombocytopenia associated with megakaryopoiesis gene variants, albeit rare, is a cause of fetal ICH

The two etiologies evoked nowadays in fetal ICH are FNAIT, when associated with a thrombocytopenia, and *COL4A1/COL4A2*, when occurring in the absence of thrombocytopenia. IPD gene screening is most often not performed unless there is a strong familial history of hemorrhage. In the rare situations where hemostasis genes are tested, this screening is most often limited to clotting factors. Indeed, this was the case for the two families with *MPL* and *MECOM* variants reported herein. In addition, information on platelet count is often missing. For some fetuses, blood count might be missing either because fetal blood sampling has not been performed or the sample had been coagulated. The identification herein of causative variants in two megakaryopoiesis genes in two distinct families strongly suggests that IPD genes should however be screened in the absence of FNAIT and *COL4A1/COL4A2* variants, even though they are rare. Indeed, these data are of major importance for genetic counseling.



The reason why only two out of the 101 probands tested for IPD gene variants were found to be mutated in this cohort is unclear. We hypothesize that, among IPD genes, only gene variants leading to a profound thrombocytopenia would lead to fetal ICH. IPD gene variants responsible of mild to moderate thrombocytopenia, might not lead to fetal ICH or might need an additional triggering event. Several additional IPD genes were however reported to be potentially responsible for severe thrombocytopenia, including THPO, PRKACG, WAS, RBM8A, GATA1 or HOXA11 genes.<sup>13,15,36</sup> Pathogenic variants of genes leading to platelet dysfunction might also contribute to fetal ICH but have not been detected in this cohort owing to their rarity. None of these genes was mutated in our cohort suggesting that their prevalence is low in fetal ICH.

### 4.3 | Clinical features of *MPL* and *MECOM* associated platelet disorders

Biallelic *MPL* pathogenic variants are known to lead to classical congenital amegakaryocytic thrombocytopenia (CAMT-MPL).<sup>37,38</sup> CAMT-MPL is a rare autosomal recessive bone marrow failure syndrome leading to pancytopenia due to a depletion of hematopoietic progenitors. CAMT-MPL diagnosis is most often made at birth or later on within the context of an isolated thrombocytopenia and a pancytopenia later on. However, clinically affected patients showed wide phenotypic variability as illustrated in a recent series of 56 patients with CAMT-MPL.<sup>39</sup> In 13 of these 56 cases, no thrombocytopenia was detected in the neonatal period. Fetal ICH was observed in only seven cases (12.5%), including four cases diagnosed retrospectively. Despite its lower frequency, ICH was the most severe manifestation. The p.R102P variant found in our study is the most frequent one in CAMT-MPL. It is encountered in most cases as a compound heterozygous variant; it has been reported only once at homozygous state in one boy with an antenatal ICH.<sup>40</sup> This variant isoform is not addressed at the cell membrane.<sup>21</sup> The p.R537W variant is far less frequent and has been reported by two previous studies neither in an ICH context. One report was a series of patients from the Choctaw tribe affected by a thrombocytopenia suggesting being a founder variant.<sup>41</sup> Regarding heterozygous relatives, Germeshausen et al.,<sup>39</sup> observed that platelet counts of heterozygous parents and siblings of probands were within the normal range except for one parent (p.R102P) with a mild thrombocytopenia. However, the possibility of a mild thrombocytopenia is contradictory with another study suggesting that germline *MPL* R102P heterozygous variant could lead to a thrombocytosis.<sup>42</sup> Altogether these data suggest that a clarification is needed about the effect of heterozygous *MPL* variants in carriers, including the father of proband F1, for proper clinical management and genetic counseling.

The second megakaryopoiesis gene (*MECOM*) plays an important role in the formation of hematopoietic stem cells and cell self-renewal. Somatic rearrangements of *MECOM* lead to an overexpression and have been reported in leukemia with a poor prognosis.<sup>43</sup> Germline missense or nonsense variants located in the zinc finger domain<sup>44</sup> lead to bone marrow failure and extra-hematological events through a haploinsufficiency mechanism.<sup>45</sup> A recent study of 12 cases with *MECOM* variants revealed a wide clinical spectrum ranging from isolated radioulnar synostosis to severe bone marrow failure without skeletal abnormalities.<sup>44</sup> Authors observed an incomplete penetrance with the presence of unaffected relatives in 4 out of 11 family members. Other abnormalities have been described such as

clinodactyly, hearing loss and cardiac malformations.<sup>46</sup> These findings were consistent with fetal examination features found in family F2 where we observed a clinical heterogeneity with one fetus having cardiac defects (a pulmonary atresia with ventricular septal defect) and no skeletal abnormality, and the other one a radio-ulnar synostosis with no cardiac defect.

Our study has some limitations. We focused our study on genes responsible of thrombocytopenia or platelet dysfunction based on physiopathological hypotheses. This study emphasizes the need for further pan genomic analyses in the next future.

To conclude, variants of *COL4A1* and *COL4A2* are the leading genetic cause of fetal ICH but genes associated with severe thrombocytopenia could be responsible of massive fetal ICH. Those genes should be investigated in ICH fetuses even in the absence of a platelet data count since the detection of such variants is crucial for genetic counseling and prenatal diagnosis. Altogether, these data emphasize the strong genetic heterogeneity of this condition and the need to perform advanced genetic analysis to broaden our knowledge on the biological mechanisms causing fetal ICH. We also emphasize the need to conduct pangenomic screening on large multicenter cohorts to better understand genotype-phenotype correlations and potentially serve as the basis for the development of targeted treatments.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

## REFERENCES

1. Monteagudo A. Intracranial hemorrhage. *Am J Obstet Gynecol.* 2020;22:B34–B37.
2. Ghi T, Simonazzi G, Perolo A, et al. Outcome of antenatally diagnosed intracranial hemorrhage: case series and review of the literature. *Ultrasound Obstet Gynecol.* 2003;22:121–130. [PubMed: 12905503]
3. Adiego B, Martínez-Ten P, Bermejo C, Estévez M, Recio Rodriguez M, Illescas T. Fetal intracranial hemorrhage. Prenatal diagnosis and postnatal outcomes. *J Matern Fetal Neonatal Med.* 2019;32:21–30. [PubMed: 28826258]
4. Bussel JB, Vander Haar EL, Berkowitz RL. New developments in fetal and neonatal alloimmune thrombocytopenia. *Am J Obstet Gynecol.* 2021;225:120–127. [PubMed: 33839095]
5. Meuwissen MEC, de Vries LS, Verbeek HA, et al. Sporadic COL4A1 mutations with extensive prenatal porencephaly resembling hydranencephaly. *Neurology.* 2011;76:844–846. [PubMed: 21357838]

6. Cavaliere AF, Turrini I, Pallottini M, et al. Genetic profiling of idiopathic antenatal intracranial haemorrhage: what we know? *Genes*. 2021;12:573. [PubMed: 33920939]
7. Hausman-Kedem M, Malinger G, Modai S, et al. Monogenic causes of apparently idiopathic perinatal intracranial hemorrhage. *Ann Neurol*. 2021;89:813–822. [PubMed: 33527515]
8. Almazni I, Stapley R, Morgan NV. Inherited thrombocytopenia: update on genes and genetic variants which may be associated with bleeding. *Front Cardiovasc Med*. 2019;6:80. [PubMed: 31275945]
9. Bastida JM, Lozano ML, Benito R, et al. Introducing high-throughput sequencing into mainstream genetic diagnosis practice in inherited platelet disorders. *Haematologica*. 2018;103:148–162. [PubMed: 28983057]
10. Downes K, Megy K, Duarte D, et al. Diagnostic high-throughput sequencing of 2396 patients with bleeding, thrombotic, and platelet disorders. *Blood*. 2019;134:2082–2091. [PubMed: 31064749]
11. Heremans J, Freson K. High-throughput sequencing for diagnosing platelet disorders: lessons learned from exploring the causes of bleeding disorders. *Int J Lab Hematol*. 2018;40:89–96. [PubMed: 29741246]
12. Johnson B, Doak R, Allsup D, et al. A comprehensive targeted next-generation sequencing panel for genetic diagnosis of patients with suspected inherited thrombocytopenia. *Res Pract Thromb Haemost*. 2018;2:640–652. [PubMed: 30349881]
13. Noris P, Pecci A. Hereditary thrombocytopenias: a growing list of disorders. *Hematology Am Soc Hematol Educ Program*. 2017;2017:385–399. [PubMed: 29222283]
14. Nurden AT, Nurden P. High-throughput sequencing for rapid diagnosis of inherited platelet disorders: a case for a European consensus. *Haematologica*. 2018;103:6–8. [PubMed: 29290630]
15. Nurden AT, Nurden P. Inherited thrombocytopenias: history, advances and perspectives. *Haematologica*. 2020;105:2004–2019. [PubMed: 32527953]
16. Seo A, Gulsuner S, Pierce S, et al. Inherited thrombocytopenia associated with mutation of UDP-galactose-4-epimerase (GALE). *Hum Mol Genet*. 2019;28:133–142. [PubMed: 30247636]
17. Shim YJ. Genetic classification and confirmation of inherited platelet disorders: current status in Korea. *Clin Exp Pediatr*. 2020;63:79–87. [PubMed: 31477680]
18. Backenroth D, Homsy J, Murillo LR, et al. CANOES: detecting rare copy number variants from whole exome sequencing data. *Nucleic Acids Res*. 2014;42:e97. [PubMed: 24771342]
19. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424. [PubMed: 25741868]
20. Hitchcock IS, Kaushansky K. Thrombopoietin from beginning to end. *Br J Haematol*. 2014;165:259–268. [PubMed: 24499199]
21. Geddis AE. Congenital amegakaryocytic thrombocytopenia. *Pediatr Blood Cancer*. 2011;57:199–203. [PubMed: 21337678]
22. de Vries LS, Koopman C, Groenendaal F, et al. COL4A1 mutation in two preterm siblings with antenatal onset of parenchymal hemorrhage. *Ann Neurol*. 2009;65:12–18. [PubMed: 19194877]
23. Lichtenbelt KD, Pistorius LR, De Tollenaer SM, Mancini GM, De Vries LS. Prenatal genetic confirmation of a COL4A1 mutation presenting with sonographic fetal intracranial hemorrhage. *Ultrasound Obstet Gynecol*. 2012;39:726–727. [PubMed: 22223490]
24. Colin E, Sentilhes L, Sarfati A, et al. Fetal intracerebral hemorrhage and cataract: think COL4A1. *J Perinatol*. 2014;34:75–77. [PubMed: 24374867]
25. Garel C, Rosenblatt J, Moutard ML, et al. Fetal intracerebral hemorrhage and COL4A1 mutation: promise and uncertainty: Letter to the Editor. *Ultrasound Obstet Gynecol*. 2013;41:228–230. [PubMed: 22811259]
26. Kutuk MS, Balta B, Koder H, et al. Is there relation between COL4A1/A2 mutations and antenatally detected fetal intraventricular hemorrhage? *Childs Nerv Syst*. 2014;30:419–424. [PubMed: 24317722]
27. Takenouchi T, Ohyagi M, Torii C, Kosaki R, Takahashi T, Kosaki K. Porencephaly in a fetus and HANAC in her father: variable expression of COL4A1 mutation. *Am J Med Genet*. 2015;167:156–158.

28. Matsumoto T, Miyakoshi K, Fukutake M, Ochiai D, Minegishi K, Tanaka M. Intracranial sonographic features demonstrating in utero development of hemorrhagic brain damage leading to schizencephaly-associated COL4A1 mutation. *J Med Ultrasonics*. 2015;42:445–446.
29. Meuwissen MEC, Halley DJJ, Smit LS, et al. The expanding phenotype of COL4A1 and COL4A2 mutations: clinical data on 13 newly identified families and a review of the literature. *Genet Med*. 2015;17:843–853. [PubMed: 25719457]
30. Khalid R, Krishnan P, Andres K, et al. COL4A1 and fetal vascular origins of schizencephaly. *Neurology*. 2018;90:232–234. [PubMed: 29282339]
31. Sato Y, Shibasaki J, Aida N, et al. Novel COL4A1 mutation in a fetus with early prenatal onset of schizencephaly. *Hum Genome Var*. 2018;5:4. [PubMed: 29760938]
32. Maurice P, Guilbaud L, Garel J, et al. Prevalence of COL4A1 and COL4A2 mutations in severe fetal multifocal hemorrhagic and/or ischemic cerebral lesions. *Ultrasound Obstet Gynecol*. 2021;57:783–789. [PubMed: 32515830]
33. Itai T, Miyatake S, Taguri M, et al. Prenatal clinical manifestations in individuals with COL4A1/2 variants. *J Med Genet*. 2021;58:505–513. [PubMed: 32732225]
34. Kuo DS, Labelle-Dumais C, Mao M, et al. Allelic heterogeneity contributes to variability in ocular dysgenesis, myopathy and brain malformations caused by Col4a1 and Col4a2 mutations. *Hum Mol Genet*. 2014;23:1709–1722. [PubMed: 24203695]
35. Jeanne M, Jorgensen J, Gould DB. Molecular and genetic analyses of collagen type IV mutant mouse models of spontaneous intracerebral hemorrhage identify mechanisms for stroke prevention. *Circulation*. 2015;131:1555–1565. [PubMed: 25753534]
36. Bouchghoul H, Quelin C, Loget P, et al. Fetal cerebral hemorrhage due to X-linked GATA1 gene mutation. *Prenat Diagn*. 2018;38:772–778. [PubMed: 29949202]
37. Ballmaier M, Germeshausen M. Congenital amegakaryocytic thrombocytopenia: clinical presentation, diagnosis, and treatment. *Semin Thromb Hemost*. 2011;37:673–681. [PubMed: 22102270]
38. Germeshausen M, Ballmaier M. Congenital amegakaryocytic thrombocytopenia – not a single disease. *Best Pract Res Clin Haematol*. 2021;34:101286. [PubMed: 34404532]
39. Germeshausen M, Ballmaier M. CAMT-MPL: congenital amegakaryocytic thrombocytopenia caused by MPL mutations - heterogeneity of a monogenic disorder - comprehensive analysis of 56 patients. *Haematologica*. 2021;106:2439–2448. [PubMed: 32703794]
40. Eshuis-Peters E, Versluys AB, Stokman MF, van der Crabben SN, Nij Bijvank SWA, van Wezel-Meijler G. Congenital amegakaryocytic thrombocytopenia type II presenting with multiple central nervous system anomalies. *Neuropediatrics*. 2016;47:128–131. [PubMed: 26854587]
41. Newman LA, Luter MA, Davis DB, Abdul-Rahman OA, Johnson JM, Megason GC. Congenital amegakaryocytic thrombocytopenia: a case series indicating 2 founder variants in the Mississippi band of Choctaw Indians. *J Pediatr Hematol Oncol*. 2017;39:573–575. [PubMed: 28697167]
42. Bellanné-Chantelot C, Mosca M, Marty C, Favier R, Vainchenker W, Plo I. Identification of MPL R102P mutation in hereditary thrombocytosis. *Front Endocrinol*. 2017;8:235.
43. Yuan X, Wang X, Bi K, Jiang G. The role of EVI-1 in normal hematopoiesis and myeloid malignancies (Review). *Int J Oncol*. 2015;47:2028–2036. [PubMed: 26496831]
44. Germeshausen M, Ancliff P, Estrada J, et al. MECOM-associated syndrome: a heterogeneous inherited bone marrow failure syndrome with amegakaryocytic thrombocytopenia. *Blood Adv*. 2018;2:586–596. [PubMed: 29540340]
45. Maki K, Yamagata T, Asai T, et al. Dysplastic definitive hematopoiesis in AML1/EVI1 knock-in embryos. *Blood*. 2005;106:2147–2155. [PubMed: 15914564]
46. Niihori T, Ouchi-Uchiyama M, Sasahara Y, et al. Mutations in MECOM, encoding oncoprotein EVI1, cause radioulnar synostosis with amegakaryocytic thrombocytopenia. *Am J Hum Genet*. 2015;97:848–854. [PubMed: 26581901]

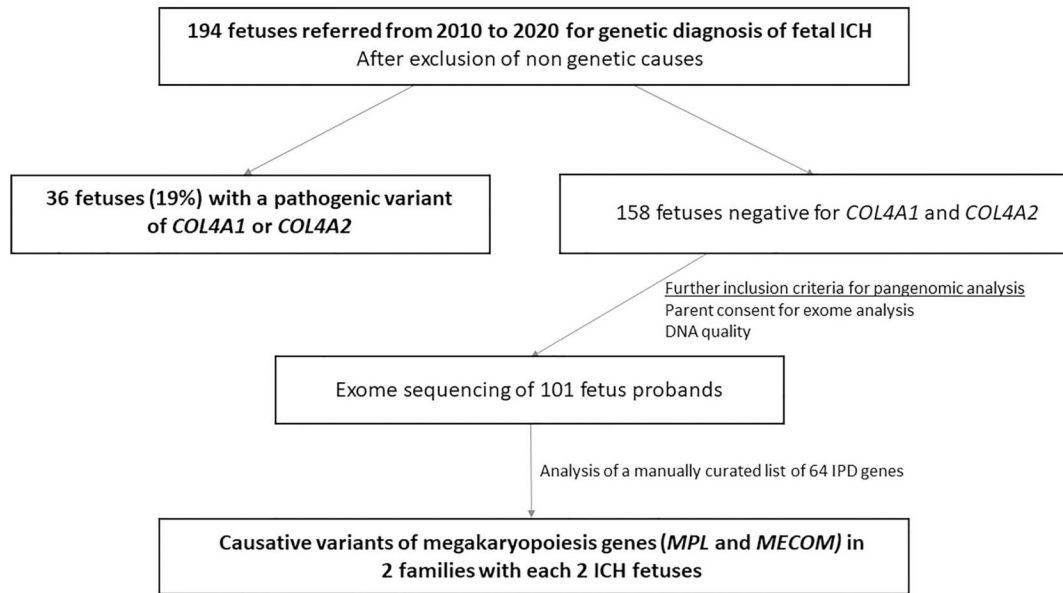
### Key points

#### What's already known about this topic?

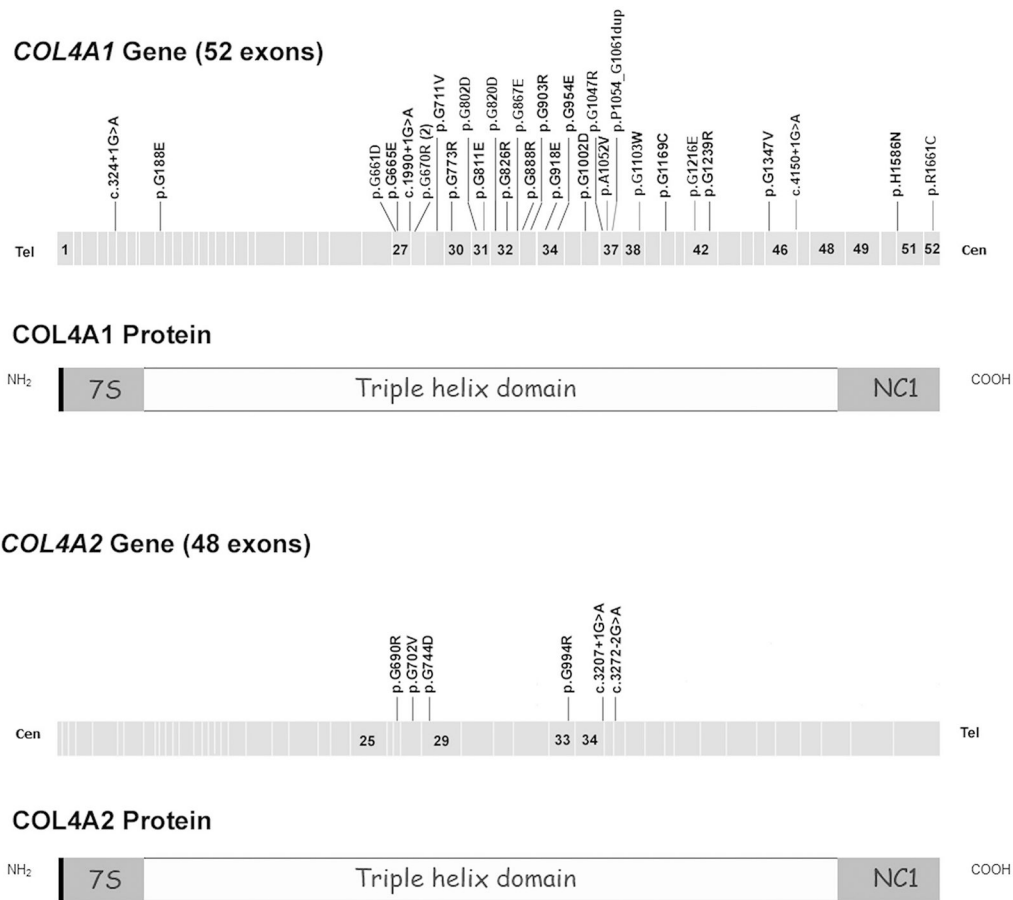
- *COL4A1/COL4A2* pathogenic variants have been reported in several fetal intracranial hemorrhage (ICH) case reports but their prevalence and characteristics in a large series of fetal ICH is lacking.
- Fetal neonatal alloimmune thrombocytopenia is a well-known cause of thrombocytopenia and ICH in infants and fetuses but very little is known regarding the role in fetal ICH of variants of inherited platelet disorder genes leading to thrombocytopenia.

#### What does this study add?

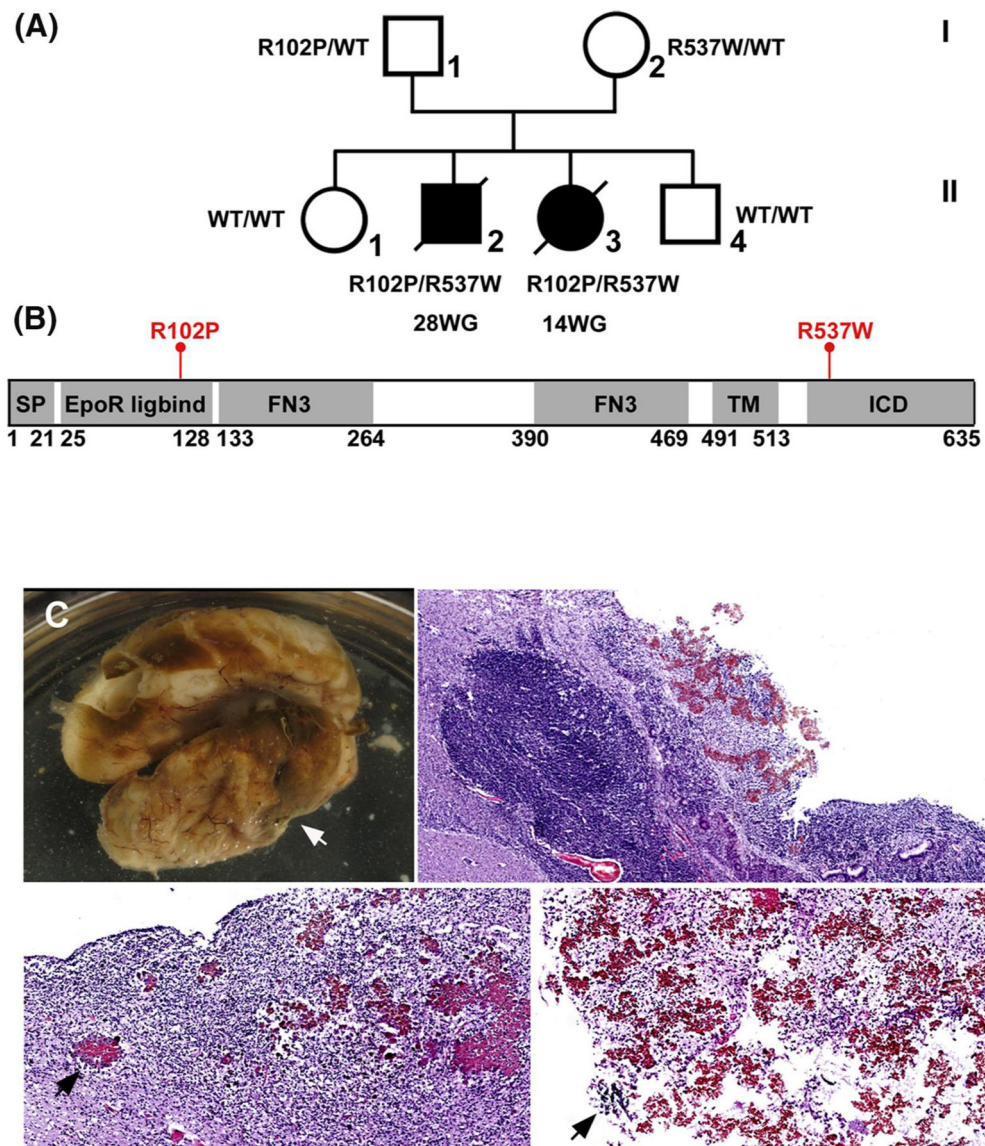
- Fetal ICH is a highly heterogeneous condition with *COL4A1/COL4A2* pathogenic variants accounting for 19% of cases with a very high de novo rate.
- Albeit rare, pathogenic variants of megakaryopoiesis genes are associated with ICH and screening of these genes should be performed in fetal ICH etiological investigation.



**FIGURE 1.**  
Study flowchart

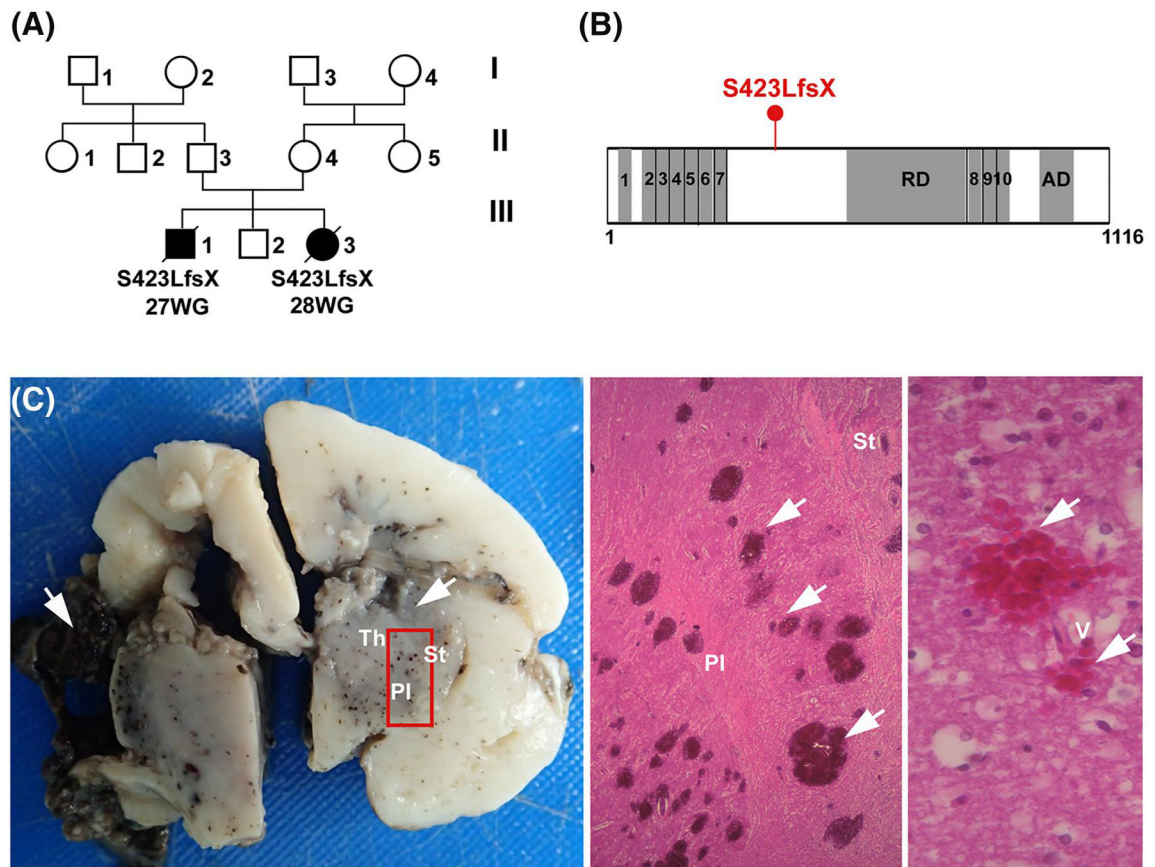


**FIGURE 2.** Distribution of *COL4A1* and *COL4A2* variants in fetal intracranial hemorrhage. Positions are annotated from the first coding nucleotide. Mature proteins are composed of three distinct domains: 7S, triple helix and non-collagenous (NC1). Cen: centromere side, Tel: telomere side



**FIGURE 3.** Family F1 with *MPL* pathogenic variants. (A) Family tree showing recessive inheritance of congenital amegakaryocytic thrombocytopenia (CAMT-MPL). Square = male; circle = female; diagonal black line = deceased individual; black filled symbol = clinically affected individual; empty symbol = clinically healthy relative. (B) Schematic representation of *MPL* protein and location of the two variants identified in the two fetuses. (C) Neuropathological findings in fetus II-2. Brain macroscopical aspect of Fetus II-2 (*top-left*). Histological examination with hematoxylin and eosin stain showing ependymal “pseudo-rosettes” (*top-right, original magnification ×40*), hemosiderin pigments (*bottom left, original magnification ×100*) and siderocalcium salts (*bottom-right, original magnification ×1000*)



**FIGURE 4.**

Family F2 with *MECOM* pathogenic variant. (A) Family tree showing dominant inheritance of *MECOM* variant. Square = male; circle = female; diagonal black line = deceased individual; black filled symbol = clinically affected individual; empty symbol = clinically healthy relative. (B) Schematic representation of EVI-I protein and location of the variant identified in the two fetuses. (C) Neuropathological findings in fetus III-3. Brain sectioning showed a disruption of the left sylvian valley parenchyma by a hematoma (*left arrow*). The brain parenchyma also appeared punctuated with multiple hemorrhagic foci (*right arrow*) and disseminated in the white and gray structures (*Th, thalamus; St, striatum; Pl, pallidum*). On a microscopic level (hematoxylin and eosin stain), these petechiae consisted of focal bleeding (*middle image*), mostly in contact with the vascular walls (*right image*)

**TABLE 1**

Main clinical and pathological features of ICH fetuses in F1 and F2 families

	Family F1		Family F2	
	II-2	II-3	III-1	III-3
<b>Case</b>	II-2	II-3	III-1	III-3
Sex	Male	Female	Male	Female
Gestation at diagnosis (weeks)	28	14	27	28
Ultrasound findings	Bilateral ventriculomegaly	Fetal death Lymphedema	Fetal death Heart failure Hydrops fetalis	Fetal death
<b>Outcome</b>	<b>TOP</b>	<b>IUFD</b>	<b>IUFD</b>	<b>IUFD</b>
Fetal Brain examination	Bilateral hydrocephaly Diffuse ventricular and subarachnoid hemorrhage	Advanced maceration Liquefied cerebral parenchyma	Hemorrhagic lesion on the right temporal area	Intraparenchymal and subarachnoid lesions encompassing the temporal area
Other autopsy anomalies	Right parenchymal necrosis Parietal cortical polymicrogyria No sign of external or visceral malformation	Bulky blood clot indicating a recent hemorrhage No sign of external or visceral malformation	Necrotic and hemorrhagic reorganizations in the left temporal area Pulmonary atresia Ventricular septal defect Overriding aorta	Diffuse intraparenchymal petechiae 11 pairs of ribs Radio-ulnar synostosis

Abbreviations: IUFD, intrauterine fetal death; TOP, termination of pregnancy.