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Prenatal exome sequencing, a powerful tool for improving the description of prenatal features associated with genetic disorders

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Abstract

Objective: Prenatal exome sequencing (pES) is now commonly used in clinical practice. It can be used to identify an additional diagnosis in around 30% of fetuses with structural defects and normal chromosomal microarray analysis (CMA). However, interpretation remains challenging due to the limited prenatal data for genetic disorders.

Method: We conducted an ancillary study including fetuses with pathogenic/likely pathogenic variants identified by trio-pES from the “AnDDI-Prenatome” study. The prenatal phenotype of each patient was categorized as typical, uncommon, or unreported based on the comparison of the prenatal findings with documented findings in the literature and public phenotype-genotype databases (ClinVar, HGMD, OMIM, and Decipher).

Results: Prenatal phenotypes were typical for 38/56 fetuses (67.9%). For the others, genotype-phenotype associations were challenging due to uncommon prenatal features (absence of recurrent hallmark, rare, or unreported). We report the first

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prenatal features associated with LINS1 and PGM1 variants. In addition, a double diagnosis was identified in three fetuses.

Conclusion: Standardizing the description of prenatal features, implementing longitudinal prenatal follow-up, and large-scale collection of prenatal features are essential steps to improving pES data interpretation.

Key points

What's already known about this topic?

- Prenatal exome sequencing detects an additional diagnosis in approximately 30% of fetuses with structural defects and normal chromosomal microarray analysis.
- Prenatal phenotyping may be challenging (limited examination of some organs, functional changes at birth, no intellectual or sensory information).
- Genetic disorders are highly heterogeneous.

What does this study add?

- In 67.9% of cases, the association between the fetal phenotype and a relevant variant was straightforward. Conversely, it was challenging in 32.1% of cases due to unreported or uncommon prenatal findings.
- We reported prenatal findings associated with bi-allelic LINS1 and PGM1 variants.
- Collaborative compilation of prenatal findings related to genetic disorders may lead to better understanding and identification of fetal conditions.

1 | INTRODUCTION

Structural defects, whether isolated or multiple, occur in approximately 2%–4% of pregnancies and are the leading cause of perinatal death (20%–25%).¹ Most of these defects are associated with genetic disorders. However, identifying a diagnosis during pregnancy is a major challenge due to clinical heterogeneity and overlap of genetic disorders. Standard karyotype and chromosomal microarray analysis (CMA), which have historically been used for prenatal genetic testing, have a diagnostic yield of approximately 30%.² Over the last decade, exome sequencing (ES) has gradually been implemented in clinical practice, particularly for investigating unsolved structural defects during pregnancy. The pooled incremental yield of prenatal ES (pES) is approximately 30% in fetuses with structural defects and negative CMA/karyotype. The diagnostic yield varies considerably depending on the phenotypic subgroup (ranging from 2% in isolated increased nuchal translucency to 53% in skeletal dysplasia) and the study design (from 15% in unselected cohorts to 42% in phenotype-selected cohorts).³ Between June 2019 and November 2021, we led the first French national multicenter pilot study (AnDDI-prenatome) aiming to implement diagnostic pES following the detection of abnormalities on ultrasound.⁴ Trio-ES identified a pathogenic/likely pathogenic variant in 52/150 fetuses (34%) with a median time to diagnosis of 28 days. The overall diagnostic yield rose to 56/150 fetuses (37%) after additional investigations: 41% (37/89) with first-line trio-pES versus 31% (19/61) with pES after normal CMA.

The implementation of pES offers new perspectives in fetal medicine, including the diagnosis of monogenic diseases, precise and personalized prenatal counselling, customization of perinatal

care, and earlier genetic counselling.⁵ However, interpreting pES may be challenging in some cases since genetic disorders have mostly been described in a postnatal setting with detailed phenotyping of affected newborns, infants, or adults. At the same time, the prenatal phenotype is limited to an indirect examination of the fetus through prenatal imaging, without any information about cognitive, sensory, or behavioral deficiencies.⁵ Despite advances in prenatal imaging, examination remains limited or impossible for some parts of the fetus. In addition, the fetal phenotype can change with gestational age and functional adaptation in the perinatal period.⁶ Overall, fetal phenotypes associated with a rare disease may differ from postnatal phenotypes, leading to the identification of uncommon or unreported findings.⁷ These factors limit the identification of pathogenic/likely pathogenic variants associated with structural defects during pregnancy and may lead to a negative result or identification of a variant of unknown significance (VUS). Confirmation or exclusion of VUS requires additional investigations. Beyond segregation analysis, functional tests used in postnatal settings are not routinely applicable during pregnancy for various reasons (access of tissue, time required, lack of normal values in unaffected fetuses...).

In this study, we investigated the clinical heterogeneity of fetal conditions in a cohort of fetuses investigated by trio-pES for structural defects. We hypothesized that the prenatal phenotype, obtained with thorough prospective phenotyping, may differ from reported data, particularly in identifying additional findings that have not yet been reported. The aim of this study was to identify uncommon or unreported prenatal features in fetuses harboring genetic disorders identified by trio-pES.

2 | MATERIAL AND METHODS

2.1 | Cohort and study design

We conducted an ancillary study of the “AnDDI-Prenatome” study, a pilot study of the French Healthcare Network for rare diseases. AnDDI-Prenatome was a prospective, collaborative multicenter study conducted between June 2019 and November 2021 (NCT03964441).⁴ In brief, trio-pES (fetus and both parents) was performed in 150 fetuses with unselected fetal structural abnormalities (at least two ultrasound abnormalities or one ultrasound abnormality known to be frequently linked to a genetic disorder). Chromosomal microarray (CMA) was conducted either before or simultaneously.

Relevant variants in known human disease genes were identified by a stepwise analysis of raw data. First, we used bioinformatics prediction tools and suspected inheritance. Secondly, a phenotype-driven analysis of the raw data was conducted using in silico targeted gene panels. All relevant variants were reviewed by a multidisciplinary team (MDT) including clinical geneticists, specialists in fetal medicine, molecular biologists, and bioinformaticians. The MDT classified each relevant variant according to the American College of Medical Genetics and Genomics (ACMG) guidelines. In all cases, a double diagnosis was systematically considered, particularly when the relevant variant did not explain all fetal features. According to the study protocol, only pathogenic/likely pathogenic variants associated with a structural defect were reported, so incidental findings and VUS were not reported. A causal diagnosis was identified in 56/150 fetuses (37%).

In this ancillary study, we enrolled patients with a causal variant identified by trio-pES in “AnDDI-Prenatome”. Patients with negative pES and VUS were not included. This study was approved by an institutional review board (Comité de Protection des Personnes (CPP)). Written informed consent was obtained from all subjects and participating family members.

2.2 | Deep-phenotyping of fetuses with a causal variant and review of prenatal findings related to genetic disorders

In “AnDDI-Prenatome”, fetal ultrasounds and MRI have always been performed by specialists in prenatal imaging within the national prenatal diagnosis network and certified by the national organization (Agence de la Biomédecine).

For this study, all fetal imaging reports (ultrasound, CT-scan, MRI) and additional biological prenatal tests (hematological, biochemical, hormonal, or cytological if performed) were blindly reviewed by a specialist in prenatal imaging (N.B.). Prenatal features were encoded using Human Phenotype Ontology (HPO) terms.

For each genetic condition diagnosed in a fetus included in this study, we performed a literature review at the time of pES interpretation to identify prenatal findings that had previously been reported. We reviewed public phenotype-genotype databases, including

ClinVar, HGMD, OMIM, and Decipher. Then, we conducted a review of the literature using a stepwise strategy in PubMed. The database was queried from inception to the date of the MDT meeting (validation of pES result during pregnancy). For the first step, search terms included “gene name or syndrome” combined with the following MeSH terms: “fetus”, “fetal”, “prenatal diagnosis”, “abnormalities, congenital”. The second step included only clinical publications selected using “gene name or syndrome”. Finally, we reviewed cohorts that have reported exome sequencing in prenatal diagnosis. Only publications in English reporting cases with molecular data were included. This literature search was performed by two authors (A.G. and N.B.).

Based on our review, we used the methodology reported by the Orphanet group, an international consortium of rare diseases experts, to classify the frequency of each prenatal finding.^{8,9} In summary, a prenatal finding was deemed common or frequent when it occurred in at least 30% of the patient population, and rare if below 30%. Then, we categorized the prenatal phenotype of each patient harboring a pathogenic/likely pathogenic variant into three groups¹: typical phenotype if the prenatal features had been previously reported and were common/frequent²; uncommon phenotype if the prenatal findings were rare; and³ unreported phenotype if the prenatal features had not been previously described. Cases with a double diagnosis were assessed separately.

3 | RESULTS

Among the 56 patients in our cohort, 59 pathogenic/likely pathogenic variants were identified, including 9 CNVs (15.3%) or aneuploidy and 50 SNVs (84.7%). Among them, three patients (5.4%) had a double diagnosis.

For the 53 fetuses with a single diagnosis, the prenatal phenotype was considered typical for 38 fetuses (71.7%), uncommon for 13 fetuses (24.5%), and unreported for 2 fetuses (3.8%). Overall, the association between prenatal phenotype and genotype was challenging for 18 out of 56 fetuses with a single or double diagnosis (32.1%).

3.1 | Fetuses with typical prenatal phenotype associated with a pathogenic/likely pathogenic variant

Most included fetuses had a typical phenotype with previously reported prenatal findings. These 38 fetuses were categorized into four phenotypic groups based on features detected by prenatal imaging: multisystem abnormalities ($n = 14$), brain malformation ($n = 11$), features suggestive of RASopathies ($n = 7$), and bone dysplasia ($n = 6$). Prenatal ES identified 32 SNVs and 6 chromosomal abnormalities confirmed by CMA performed in parallel: mosaic trisomy 14, mosaic trisomy 17, trisomy 18, tetrasomy 12p, 16p13.3 microdeletion, and a de novo supernumerary marker 15q11.1q11.2. In all cases, the association between prenatal phenotype and genotype was

not difficult to identify. Prenatal findings and molecular data are summarized in Table 1.

3.2 | Fetuses with an uncommon prenatal phenotype associated with a pathogenic/likely pathogenic variants

The prenatal phenotype was considered uncommon in 13 fetuses affected by neurodevelopmental disorders, syndromic or not, with an evolving phenotype after birth. Although the fetal phenotype was uncommon, the relevant variant was identified by *in silico* prediction tools and suspected inheritance.

The fetal phenotype was uncommon for 2 fetuses without recurrent hallmarks commonly observed in postnatal settings. We identified a heterozygous *de novo* ASXL1 truncating variant (NM_015338.6:c.1934dup) leading to Bohring-Opitz syndrome (BOS, MIM#605039) in a male fetus (fetus II.1) with corpus callosum dysgenesis, cerebellar hypoplasia, and shortened long bones. BOS is characterized by intellectual disability (ID), severe intrauterine growth restriction (IUGR), distinctive craniofacial features and trigonocephaly/microcephaly.¹⁰ Although IUGR and microcephaly were absent in this fetus, previous reports in postnatal cohorts have documented brain abnormalities (corpus callosum and cerebellar abnormalities).^{11–13} In another male fetus (fetus II.2) with increased nuchal translucency (NT), short corpus callosum, olfactory lobe agenesis, and webbed neck, we identified a heterozygous *de novo* ANKRD11 missense variant (NM_013275.5:c.3931C>T). While brain abnormalities are reported in approximately 50% of patients with KBG syndrome (MIM#148050), corpus callosum and olfactory nerve/bulb abnormalities affect only 5%. The fetus did not display the skeletal abnormalities commonly associated with KBG syndrome. Identification of distinctive craniofacial findings is limited during pregnancy. Additionally, an early onset with increased NT has been reported in one case.¹⁴ For both fetuses, the prenatal findings had previously been reported as rare features in postnatal cohorts.

For our other cases, the identified prenatal findings were either not reported or rarely documented in the literature. This suggests or supports a broadening of the clinical spectrum of genetic disorders. We identified an early fetal hydrops in a female fetus (II.3) with a heterozygous *de novo* GNB2 missense variant (NM_005273.4: c.265A>G). GNB2 variants are involved in a neurodevelopmental disorder with hypotonia at birth, facial dysmorphism and developmental delay (MIM#619503). So far, only one fetus, harboring the same c.265A>G variant has been reported with facial features, congenital heart defect (CHD), renal hypoplasia, hyperechoic bowel, single umbilical artery, asymmetric growth and decreased fetal movements.¹⁵ Our observations suggest an early and severe phenotype related to a *de novo* GNB2 missense variant. A *de novo* PPM1D variant (NM_003620.3: c.1372C>T) involved in Jansen-de Vries syndrome (JDVS, #617450) was identified in fetus II.4, who had multisystem abnormalities (Pierre-Robin sequence (PRS), coarctation of aorta, abnormal sacral segmentation, vertebral and rib fusion, delayed skeletal maturation, and

abnormal cerebellar vermis). JDVS is characterized by findings that cannot be detected prenatally (developmental delay, ID, and behavioral abnormalities). Short stature at birth (<-2 SD) with shortened long bones has been reported in some cases. Therefore, we reported a severe phenotype related to a PPM1D variant.

We identified enlarged kidneys in a large-for-gestational-age fetus (II.5) with a 22q11.2 deletion. 22q11.2 deletion syndrome includes a wide spectrum of congenital heart defects, palatal abnormalities, immune deficiency, characteristic facial features, and learning difficulties. The most common kidney abnormalities related to the 22q11.2 deletion spectrum are multicystic dysplasia.^{16–18} Finally, we identified a 17q25.3 deletion in fetus II.6 with ventricular septal defect, IUGR and PRS. CHD has been reported in del17q25.3 deletion, whereas IUGR and PRS have not.¹⁹

For cases II.7 to II.13, comparisons with published data were limited due to the small number of reported fetal cases. Clinical and biological data are summarized in Table 2.

3.3 | Fetuses with unreported prenatal phenotype associated with a pathogenic/likely pathogenic variant

Despite the lack of prenatal description, a pathogenic/likely pathogenic variant was identified in two fetuses thanks to *in silico* prediction tools and suspected inheritance. We therefore reported the early prenatal onset of well-known pediatric disorders, as described in Table 3.

We identified bi-allelic LINS1 truncating variants (NM_001040616.3: c.597delA and c.557_558delAA) in a male fetus (IV.1) with increased nuchal translucency, IUGR and asymmetric cerebral ventricles. Bi-allelic LINS1 variants (truncating or missense) have previously been involved in an ultra-rare autosomal recessive ID (MIM#614330), syndromic or not, with autistic features, schizophrenia and anxiety.^{20,21}

Bi-allelic PGM1 truncating variants (NM_002633.3: c.423delA and c.157_158delinsG) were identified in a male fetus (IV.2) with increased NT, polyhydramnios, PRS and shortened long bones. Bi-allelic PGM1 variants (MIM#171900) are associated with a congenital disorder of glycosylation characterized by abnormalities in glycogen metabolism, glycolysis, and protein glycosylation (PGM1-CDG, MIM# 614921). Congenital malformations including bifid uvula, cleft palate, micrognathia and PRS have been reported in postnatal cohorts following neonatal diagnosis.²² A prenatal onset with airway compression leading to polyhydramnios was not previously reported. The age of onset and severity of PGM1-CDG are highly variable and may be linked to the residual PGM1 enzyme activity.²²

3.4 | Fetuses with double diagnosis

Three fetuses received a double diagnosis: two fetuses with two SNVs and one fetus combining one CNV and one SNV.

TABLE 1 Clinical and molecular data of patients with typical phenotype.

Patient group	Phenotypic group	GA at first ultrasound feature (WG)	Ultrasound feature (1)	GA at pES	Gene (OMIM)	Phenotype (OMIM)	Pregnancy outcome	Reason of parental decision-making
I.1	Bone dysplasia	12	Bilateral talipes equinovarus (HP:0001776) Tibial bowing (HP:0002982) Fibular bowing (HP:0002982)	26	COL1A2 (120160)	Osteogenesis imperfecta type II, III, IV (166210, 259420, 166220)	COP after result	Favorable prognosis on positive ES results
I.2	Bone dysplasia	20	Short proximal long bones (HP:0003026) Short thorax (HP:0010306) Short ribs (HP:0000773) Squared iliac bones (HP:0003177) Dysplastic iliac wing (HP:0008794) Accelerated skeletal maturation (HP:0005616)	30	DYNC2H1 (603297)	Short-rib thoracic dysplasia 3 with or without polydactyly (613091)	COP after result	Favorable prognosis on scan signs and negative ES results
I.3	Bone dysplasia	22	Femoral bowing (HP:0002980) Short hands (HP:0004279) Short ribs (HP:0000773)	24	FGFR3 (134934)	Thanatophoric dysplasia type I, II (187600, 187601)	COP after result	Favorable prognosis on positive ES results
I.4	Bone dysplasia	20	Corpus callosum agenesis (HP:0001274)	21	NFIA (600727)	Brain malformations with or without urinary tract defects (613735)	TOP after result	Poor prognosis on ES diagnosis
I.5	Bone dysplasia	21	Bilateral talipes equinovarus (HP:0001776) Micomelia (HP:0002983) Platyspondyly (HP:0000926)	24	SLC26A2 (606718)	Achondrogenesis Ib (600972), Atelosteogenesis, type II (256050), Diastrophic dysplasia (222600), Diastrophic dysplasia, broad bone-platyspondylic variant (222600)	TOP after result	Poor prognosis on ES diagnosis
I.6	Bone dysplasia	31	Choroid plexus cyst (HP:0002190) Hand oligodactyly (HP:0001180) Short proximal long bones (HP:0003026)	31	TBX3 (601621)	Ulnar-mammary syndrome (181450)	COP after result	Favorable prognosis on positive ES results
I.7	Brain malformation	19	Ventriculomegaly (HP:0002119) Schizencephaly (HP:0010636) Abnormal corpus callosum morphology (HP:0001273) Abnormal atrioventricular valve morphology (HP:0006705) Abnormal external genitalia (HP:0000811)	21	COL4A1 (120130)	Microangiopathy and leukoencephalopathy, pontine, autosomal dominant (618564)	TOP after result	Poor prognosis on ES diagnosis

(Continues)

TABLE 1 (Continued)

Patient group	Phenotypic group	GA at first ultrasound feature (WG)	Ultrasound feature (1)	GA at pES	Gene (OMIM)	Phenotype (OMIM)	Pregnancy outcome	Reason of parental decision-making
			Abnormal periventricular white matter morphology (HP:0002518)					
			Cerebellar hypoplasia (HP:0001321)					
			Perisylvian polymicrogyria (HP:0012650)					
I.8	Brain malformation	15	Corpus callosum agenesis (HP:0001274)	18	COL4A1 (120130)	Microangiopathy and leukoencephalopathy, pontine, autosomal dominant (618564)	TOP after result	Poor prognosis on ES diagnosis
			Ventriculomegaly (HP:0002119)					
			Porencephalic cysts (HP:0002132)					
I.9	Brain malformation	20	Corpus callosum agenesis (HP:0001274)	21	CREBBP (600140)	Rubinstein-Taybi syndrome 1 (180849)	TOP after result	Poor prognosis on ES diagnosis
I.10	Brain malformation	30	Cerebellar hypoplasia (HP:0001321)	29	DLL1 (606582)	Neurodevelopmental disorder with nonspecific brain abnormalities and with or without seizures (618709)	COP after result	Parental choice despite poor prognosis on positive ES results
			Ventriculomegaly (HP:0002119)					
			Enlarged fetal cisterna magna (HP:0011427)					
I.11	Brain malformation	20	Corpus callosum agenesis (HP:0001274)	25	SMC 15q11.2		TOP before result	Poor prognosis on CMA diagnosis
I.12	Brain malformation	20	Ventriculomegaly (HP:0002119)	20	L1CAM (308840)	Hydrocephalus, congenital, X-linked (307000)	TOP after result	Poor prognosis on ES diagnosis
			Abnormal septum pellucidum (HP:0007375)					
			Corpus callosum hypoplasia (HP:0007370)					
I.13	Brain malformation	20	Increased nuchal translucency (HP:0010880)	24	MTOR (601231)	Smith-Kingsmore syndrome (616638)	COP after result	Uncertain prognosis on positive ES result
			Macrocephaly (HP:0000256)					
			Hypertelorism (HP:0000316)					
			Hepatomegaly (HP:0002240)					
			Ventriculomegaly (HP:0002119)					
I.14	Brain malformation	22	Congenital cataract (HP:0000519)	23	NHS (300457)	Nance-Horan syndrome (302350)	TOP after result	Poor prognosis on ES diagnosis
			Multicystic kidney dysplasia (HP:00000003)					
			Single umbilical artery (HP:0001195)					

TABLE 1 (Continued)

Patient group	Phenotypic group	GA at first ultrasound feature (WG)	Ultrasound feature (1)	GA at pES	Gene (OMIM)	Phenotype (OMIM)	Pregnancy outcome	Reason of parental decision-making
I.15	Brain malformation	20	Corpus callosum agenesis (HP:0001274) Microretrognathia (HP:0000308) Foot malposition (HP:0005656)	21	SCYL2 (616365)	Arthrogryposis multiplex congenita 4, neurogenic, with agenesis of the corpus callosum (618766)	TOP before result	Poor prognosis on ultrasound signs
I.16	Brain malformation	24	Partial corpus callosum agenesis (HP:0001338) Abnormal cortical gyration (HP:0002536) Pontocerebellar atrophy (HP:0006879)	24	TUBB (191130)	Cortical dysplasia, complex, with other brain malformations 6 (615771)	TOP before result	Poor prognosis on brain MRI signs
I.17	Brain malformation	21	Corpus callosum agenesis (HP:0001274) Unilateral cleft lip and palate (HP:0000202) Lobar holoprosencephaly (HP:0006870) Abnormal cortical gyration (HP:0002536)	22	FGF8 (600483)	Hypogonadotropic hypogonadism 6 with or without anosmia (612702)	TOP after result	Poor prognosis on brain MRI signs
I.18	Multisystem anomalies	23	Increased nuchal translucency (HP:0010880) Polyhydramnios (HP:0001561) Hypertelorism (HP:0000316) Unilateral cleft lip and palate (HP:0000202) Enlarged fetal cisterna magna (HP:0011427) Persistent left superior vena cava (HP:0005301) Hyperechogenic kidneys (HP:0004719) Bilateral enlarged kidney (HP:0000105) Bilateral fetal pyelectasis (HP:0000105)	22	ACTB (102630)	Baraitser-Winter syndrome 1 (243310)	TOP after result	Poor prognosis on ES diagnosis
I.19	Multisystem anomalies	22	Widened cerebral subarachnoid space (HP:0012766) Duplication of phalanx of hallux (HP:0010066) Abnormal cortical gyration (HP:0002536) Hypoplasia of the cerebellar vermis (HP:0006817)	25	del16p13.3		TOP before result	Poor prognosis on brain MRI signs
I.20	Multisystem anomalies	24	Oligohydramnios (HP:0001562) Bilateral renal dysplasia (HP:0012582) Bilateral renal hypoplasia (HP:0012584)	26	EYA1 (601653)	Branchiootorenal syndrome 1, with or without cataracts (113650)	TOP after result	Poor prognosis on ES diagnosis

(Continues)

TABLE 1 (Continued)

Patient	Phenotypic group	GA at first ultrasound feature (WG)	Ultrasound feature (1)	GA at pES	Gene (OMIM)	Phenotype (OMIM)	Pregnancy outcome	Reason of parental decision-making
I.21	Multisystem anomalies	10	Increased nuchal translucency (HP:0010880) Hydrops fetalis (HP:0001789)	21	GUSB (611499)	Mucopolysaccharidosis VII (253220)	TOP after result	Poor prognosis on ES diagnosis
I.22	Multisystem anomalies	30	Intrauterine growth retardation (HP:0001511) Microcephaly (HP:0000252) Hypotelorism (HP:0000601)	30	IGF1R (147370)	Resistance to Insulin-like growth factor I (270450)	COP after result	Favorable prognosis on positive ES results
I.23	Multisystem anomalies	20	Hypertelorism (HP:0000316) Retrognathia (HP:0000278) Patent foramen ovale (HP:0001655) Bilateral fetal pyelectasis (HP:0000105)	26	KAT6B (605880)	SBBYSS syndrome (603736)	TOP after result	Poor prognosis on ES diagnosis
I.24	Multisystem anomalies	10	Cystic hygroma (HP:0000476) Intrauterine growth retardation (HP:0001511) Bilateral cleft lip and palate (HP:0002744) Bilateral fetal pyelectasis (HP:0000105)	24	KMT2D (602113)	Kabuki syndrome 1 (147920)	TOP before result	Poor prognosis on ultrasound signs
I.25	Multisystem anomalies	10	Cystic hygroma (HP:0000476) Hypertelorism (HP:0000316) Unilateral cleft palate (HP:0000202) Complex heart defect (HP:0001627) Unilateral cystic kidney (HP:0000003) Postaxial polydactyly (HP:0100259)	18	KMT2D (602113)	Kabuki syndrome 1 (147920)	TOP before result	Poor prognosis on ultrasound signs
I.26	Multisystem anomalies	20	Bilateral talipes equinovarus (HP:0001776) Blake's pouch cyst (HP:0033140)	21	Mosaic trisomy 17		TOP before result	Poor prognosis on CMA diagnosis
I.27	Multisystem anomalies	20	Intrauterine growth retardation (HP:0001511) Tetralogy of Fallot (HP:0001636) Hypercholeic bowel (HP:0010943) Abnormality of the gallbladder (HP:0005264)	23	Mosaic trisomy 14		TOP after result	Poor prognosis on ES diagnosis

TABLE 1 (Continued)

Patient group	Phenotypic group	GA at first ultrasound feature (WG)	Ultrasound feature (1)	GA at pES	Gene (OMIM)	Phenotype (OMIM)	Pregnancy outcome	Reason of parental decision-making
I.28	Multisystem anomalies	21	Intrauterine growth retardation (HP:0001511) Brachycephaly (HP:0000248) Abnormal facial shape (HP:0001999) Partial corpus callosum agenesis (HP:0001338) Cerebellar vermis hypoplasia (HP:0001320) Congenital heart defect (HP:0001627) Single umbilical artery (HP:0001195)	21	NIPBL (608667)	Cornelia de Lange syndrome 1 (122470)	TOP after result	Poor prognosis on ES diagnosis
I.29	Multisystem anomalies	20	Polyhydramnios (HP:0001561) Ventriculomegaly (HP:0002119) Abnormal vertebral column (HP:0000925) Hypospadias (HP:0000047) Postaxial hand polydactyly (HP:0001162) Absent septum pellucidum (HP:0001331) Lumbar spinal canal stenosis (HP:0004610) Abnormal vertebral segmentation and fusion (HP:0005640)	21	SMO (601500)	Pallister-Hall-like syndrome (241800)	COP after result	Parental choice despite poor prognosis on positive ES results
I.30	Multisystem anomalies	31	Polyhydramnios (HP:0001561) Flat face (HP:0012368) Cleft palate (HP:0000175) Absence of stomach bubble on fetal sonography (HP:0010963) Depressed nasal bridge (HP:0005280)	30	Tetrasomy 12p		TOP after result	Poor prognosis on ES diagnosis
I.31	Multisystem anomalies	20	Hypotelorism (HP:0000601) Congenital diaphragmatic hernia (HP:0000776) Cerebellar vermis hypoplasia (HP:0001320) Hypoplasia of the pons (HP:0012110) Abnormal cortical gyration (HP:0002536)	28	Trisomy 18		TOP before result	Poor prognosis on CMA diagnosis

(Continues)

TABLE 1 (Continued)

Patient group	Phenotypic group	GA at first ultrasound feature (WG)	Ultrasound feature (1)	GA at pES	Gene (OMIM)	Phenotype (OMIM)	Pregnancy outcome	Reason of parental decision-making
I.32	RASopathies	20	Polyhydramnios (HP:0001561) Large for gestational age (HP:0001520) Pleural effusion (HP:0002202) Bilateral enlarged kidney (HP:0000105) Hepatomegaly (HP:0002240) Splenomegaly (HP:0001744)	29	BRAF (164757)	LEOPARD syndrome 3 (613707), Noonan syndrome 7 (613706)	TOP after result	Poor prognosis on ES diagnosis
I.33	RASopathies	16	Polyhydramnios (HP:0001561) Large for gestational age (HP:0001520) Hepatomegaly (HP:0002240) Abnormal foot morphology (HP:0001760)	20	HRAS (190020)	Costello syndrome (218040)	TOP before result	Poor prognosis on ultrasound signs
I.34	RASopathies	10	Cystic hygroma (HP:0000476) Polyhydramnios (HP:0001561) Large for gestational age (HP:0001520) Low-set ears (HP:0000369)	30	NRAS (164790)	Noonan syndrome 6 (613224)	COP after result	Favorable prognosis on positive ES results
I.35	RASopathies	19	Increased nuchal translucency (HP:0010880) Chylothorax (HP:0025677) Pleural effusion (HP:0002202) Edema (HP:0000969) Brachydactyly (HP:0001156) Unilateral renal agenesis (HP:0000122)	23	PTPN11 (176876)	Noonan syndrome 1 (163950), LEOPARD syndrome 1 (151100)	TOP before result	Poor prognosis on ultrasound signs
I.36	RASopathies	20	Polyhydramnios (HP:0001561) Brachycephaly (HP:0000248) Retrognathia (HP:0000278) Complex heart defect (HP:0001627) Bilateral talipes equinovarus (HP:0001776)	26	PTPN11 (176876)	Noonan syndrome 1 (163950), LEOPARD syndrome 1 (151100)	TOP before result	Poor prognosis on ultrasound signs

TABLE 1 (Continued)

Patient	Phenotypic group	GA at first ultrasound feature (WG)	Ultrasound feature (1)	GA at pES	Gene (OMIM)	Phenotype (OMIM)	Pregnancy outcome	Reason of parental decision-making
I.37	RASopathies	10	Polyh cystic hygroma (HP:0000476) Polyhydramnios (HP:0001561) Large for gestational age (HP:0001520) Bilateral enlarged kidney (HP:0000105) Bilateral fetal pyelectasis (HP:0000105)	29	SOS1 (182530)	Noonan syndrome 4 (610733)	TOP before result	Poor prognosis on ultrasound signs
I.38	RASopathies	20	Polyhydramnios (HP:0001561) Large for gestational age (HP:0001520) Macroglossia (HP:0000158) Renal hypoplasia (HP:0012584) Hepatomegaly (HP:0002240) Pleural effusion (HP:0002202)	29	SOS1 (182530)	Noonan syndrome 4 (610733)	COP after result	Favorable prognosis on positive ES results

Abbreviations: COP, continuation of pregnancy; F, female fetus; GA, gestational age; M, male fetus; pES, prenatal exome sequencing; SMC, Supernumerary marker chromosome; TOP, termination of pregnancy; WG, weeks of gestation.

TABLE 2 Clinical and molecular data of patients with uncommon or unreported phenotype.

Case	Sex	GA of first US abnormality (WG)	Phenotype (HPO term)	GA at pES results (WG)	Pregnancy outcome	Gene (OMIM number)	Variation cDNA	Inheritance	Related phenotype (OMIM number)	Absence of recurrent hallmarks	Rare features	Unreported features
II.1	M	31	Corpus callosum dysgenesis (HP:0001273) Cerebellar hypoplasia (HP:0001321) Shortened long bones (HP:0003026)	33	TOP before ES result	ASXL1 (612990)	NM_015338.6:c.1934dup	De novo	Bohring-Opitz syndrome (#605039)	IUGR Microcephaly	Brain abnormalities reported in postnatal cohorts Prenatal CC agenesis (1 patient)	-
II.2	M	22	Increased nuchal translucency (HP:0010880) Short corpus callosum (HP:0200012) Olfactory lobe agenesis (HP:0001341) Webbed neck (HP:0000465)	26	TOP after result	ANKRD11 (611192)	NM_013275.5:c.3931C>T	De novo	KBG syndrome (#148050)	Distinctive craniofacial findings Short stature Skeletal abnormalities	CC morphology and olfactory nerve/bulb abnormalities in 5% of cases Increased nuchal translucency, IUGR, polyhydramnios (1 patient)	-
II.3	F	12	Hydrops (HP:0001789)	19	Spont. Abortion	GNB2 (139390)	NM_005273.4:c.265A>G	De novo	Neurodevelopmental disorder with hypotonia and facial dysmorphism (#619503)	Hypotonia congenital heart defect Nonspecific renal abnormalities Joint contractures or hyperextensibility	-	Hydrops
II.4	F	20	Pierre-Robin sequence (HP:0000201) Coarctation of aorta (HP:0001680) Abnormal sacral segmentation (HP:0008468) Vertebral fusion (HP:0002948) Rib fusion (HP:0000902)	30	TOP after result	PPM1D (605100)	NM_003620.3:c.1372C>T	De novo	Jansen-de Vries syndrome (#617450)	Psychomotor development, intellectual disability, speech delay, and behavioral abnormalities. Most patients have feeding and gastrointestinal difficulties, high pain threshold and/or hypersensitivity to sound, and facial features.	-	Pierre-Robin sequence coarctation of aorta Abnormal sacral segmentation Vertebral fusion Rib fusion Delayed skeletal maturation Abnormal cerebellar vermis

TABLE 2 (Continued)

Case	Sex	GA of first US abnormality (WG)	Phenotype (HPO term)	GA at pES results (WG)	Pregnancy outcome	Gene (OMIM number) Variation cDNA Inheritance Related phenotype (OMIM number)	Absence of recurrent hallmarks	Rare features	Unreported features
II.5	F	20	Delayed skeletal maturation (HP:0002750) Abnormal cerebellar vermis (HP:0002334)	24	TOP before ES result	del22q11.2 2kb: (18893886–21386103)x1 De novo 22q11.2 deletion syndrome	Congenital heart defect Palate abnormalities	-	Enlarged kidneys
II.6	F	21	IUGR (HP:0001511) Pierre-Robin sequence (HP:0000201) Ventricular septal defect (HP:0001629)	23	COP	del17q25.3 1.5 Mb: (79539041–81052322)x1 De novo 17q25.3 deletion syndrome	-	-	Pierre-Robin sequence IUGR
II.7	M	22	Abnormal corpus callosum (HP:0001273) Bilateral talipes equinovarus (HP:0001776)	25	COP	ZNF148 (601897) NM_021964.3:c.1624C>T De novo Global developmental delay, absent or hypoplastic corpus callosum, and facial dysmorphism (#601897)	-	Abnormal corpus callosum (1/4) Bilateral talipes equinovarus (1/4) Hydronephrosis (1/4) Cystic renal dysplasia (2/4) Ventriculomegaly (1/4) Hypoplastic left heart (1/4)	-
II.8	M	24	Cerebellar hypoplasia (HP:0007360) Unilateral talipes equinovarus (HP:0001762) Olivopontocerebellar hypoplasia (HP:0006955) Widened subarachnoid space (HP:0012704)	32	TOP after ES result	TSEN54 (608755) NM_207346.2:c.919G>T and c.950delC Compound heterozygous Pontocerebellar hypoplasia type 2A (#277470), 4 (#225753) and 5 (#610204)	-	Abnormal fetal movement (3/14) Cerebellar hypoplasia (2/14) Arthrogryposis multiplex congenita (2/14) Polyhydramnios (3/14) No prenatal findings (7/14)	Olivopontocerebellar hypoplasia Widened subarachnoid space

(Continues)

TABLE 2 (Continued)

Case	Sex	GA of first US abnormality (WG)	Phenotype (HPO term)	GA at pES results (WG)	Pregnancy outcome	Gene (OMIM number) Variation cDNA Inheritance Related phenotype (OMIM number)	Absence of recurrent hallmarks	Rare features	Unreported features
II.9	?	20	Corpus callosum hypoplasia (HP:0007370) Choroid plexus cysts (HP:0011426) Congenital heart defect (HP:0001627)	24	TOP before result	MRP522 (605810) NM_020191.2:c.480_481insA and c.509G>A Compound heterozygous Combined oxidative phosphorylation deficiency 5 (#611719)	-	Tetralogy of Fallot (1/8) Hypertrophic cardiomyopathy (1/8) Microcephaly (1/8) Abnormal cerebral ventricle morphology (1/8) Dysplastic corpus callosum (3/8) No prenatal findings (5/8)	Choroid plexus cysts
II.10	?	25	Abnormal corpus callosum morphology (HP:0001273) Ventriculomegaly (HP:0002119) Abnormal cortical gyration (HP:0002536)	29	TOP before result	GRIN2B (138252) NM_000834.3:c.2684_2685del De novo Developmental and epileptic encephalopathy 27 (#616139) Intellectual developmental disorder, autosomal dominant 6, with or without seizures (#613970)	-	Agnesis of corpus callosum (1/6) CAKUT (1/6) No prenatal findings (4/6)	Abnormal cortical gyration
II.11	?	12	Facial edema (HP:0000282) Edema of the lower limbs (HP:0010741) Hyperechoic bowel (HP:0010943)	25	COP	FLT4 (136352) NM_182925.4:c.3332G>A De novo Lymphatic malformation 1 (#153100)	-	Increased nuchal translucency (1/3) Hydrops fetalis (1/3) Pleural effusion (1/3) Edema of the lower limbs (1/3)	-
II.12	M	18	Microcephaly (HP:0000252) Bilateral cataract (HP:0000519) Pierre-Robin sequence (HP:0000201) Horseshoe kidney (HP:0000085)	22	TOP after result	NUP188 (615587) NM_015354.2:c.3515+1G>A and c.1851_1852delinsG Compound heterozygous Sandestig-Stefanova syndrome (#618804)	-	Intrauterine growth retardation (1/2) Microcephaly (1/2) Decreased fetal movement (1/2)	Bilateral cataract Pierre-Robin sequence Horseshoe kidney

TABLE 2 (Continued)

Case	Sex	GA of first US abnormality (WG)	Phenotype (HPO term)	GA at pES results (WG)	Pregnancy outcome	Gene (OMIM number)	Variation cDNA	Inheritance	Related phenotype (OMIM number)	Absence of recurrent hallmarks	Rare features	Unreported features
II.13	M	10	Cystic hygroma (HP:0000476) Abnormal cerebral ventricle morphology (HP:0002118) Postaxial hand polydactyly (HP:0001162)	20	TOP after result	MYCN (164840)	NM_005378.4:c.179C>T	De novo	MYCN GoF syndrome	-	Ventriculomegaly (1/1) Postaxial hand polydactyly (1/1) Macrocephaly (1/1)	Cystic hygroma

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, Autism spectrum disorder; CAKUT, congenital anomalies of the kidney and urinary tract; COP, continuation of pregnancy; F, female fetus; GA, gestational age; GoF, gain of function; ID, intellectual disability; IUFG, intrauterine growth restriction; M, male fetus; TOP, termination of pregnancy; WG, weeks of gestation.

We identified a 17q12 microdeletion associated with a GREB1L variant (NM_001142966.1:c.2252G>A) in a fetus with polyhydramnios, esophageal atresia, renal agenesis and a single umbilical artery. These prenatal features were previously reported in 17q12 recurrent microdeletion syndrome. Kidney abnormalities could be related to both variants. Indeed, GREB1L variants are associated with renal hypoplasia/aplasia (MIM#617805) and the GREB1L variant identified in our patient was reported as likely pathogenic in the ClinVar database. It was inherited from the mother and present in the mother's sister, who was both asymptomatic. In addition, the couple's previous pregnancy was complicated by IUFD at 15 weeks of gestation. IUFD and early neonatal death have also been reported in renal hypoplasia/aplasia related to a GREB1L variant.

We identified a homozygous EFEMP2 missense (NM_016938.4:c.379T>C) and a homozygous RAG1 nonsense (NM_000448.3:c.1767C>G) in a male fetus with decreased fetal movements, prefrontal edema, retrognathia, small stomach, complex CHD (dilated tortuous aorta, hypoplastic proximal pulmonary arteries and thickened ventricular walls), and external genital hypoplasia. Both variants, inherited from consanguineous parents, were previously reported as likely pathogenic in the ClinVar database. The prenatal phenotype was partially compatible with previously reported prenatal presentations secondary to EFEMP2 biallelic variants. Biallelic RAG1 truncating variants have been implicated in severe combined immunodeficiency, which is undetectable during pregnancy.

In the last fetus, who had an isolated cystic hygroma, we identified a homozygous ASCC1 variant and a homozygous truncating CSPP1 variant. Cystic hygroma has been reported in the early onset of spinal muscular atrophy with congenital bone fractures related to biallelic ASCC1 variants. In addition, biallelic CSPP1 variants are involved in Joubert syndrome (MIM#615636). However, due to the early onset, the recurrent hallmarks of JS were undetectable.

4 | DISCUSSION

In this study, we report previously reported, uncommon and previously unreported prenatal findings identified in a prospective study that offered pES for fetal structural abnormalities. Our results suggest that the widespread implementation of pES in routine diagnosis would be a powerful way to enhance our knowledge regarding prenatal phenotypes and the natural history of genetic disorders.

4.1 | Rapid identification of most diagnoses

In most of our patients (67.9%), the prenatal features observed matched those commonly reported in databases and the literature. In these instances, interpreting the association of relevant variants identified by pES with a prenatal phenotype was straightforward. Our results confirm that implementing pES in routine diagnosis for the investigation of fetal structural abnormalities should not be hindered

TABLE 3 Clinical and molecular data of patients with unreported prenatal phenotype.

Case	Sex	GA of first US abnormality (WG)	Phenotype (HPO term)	GA at pES results (WG)	Pregnancy outcome	Gene (OMIM number) Variation cDNA Inheritance Related phenotype (OMIM number)	Postnatal data reported
III.1	F	22	Increased nuchal translucency (HP:0010880) IUGR (HP:0001511) Asymmetric cerebral ventricles (HP:0002118)	27	COP	LINS1 (610350) NM_001040616.3:c.597del and c.557_558del Compound heterozygous intellectual developmental disorder, autosomal recessive 27 (#614340)	Intellectual disability (HP:0001249) Schizophrenia (HP:0100753) Anxiety (HP:0000739) Speech impairment (HP:0000750) Dystonia (HP:0001332) Abnormal eye movements (HP:0000496) Craniofacial features (HP:0001999)
III.2	M	12	Increased nuchal translucency (HP:0010880) Polyhydramnios (HP:0001561) Pierre-Robin sequence (HP:0000201) Shortened long bones (HP:0003026)	27	TOP after ES result	PGM1 (171900) NM_002633.3:c.423del and c.157_158delinsG Compound heterozygous Congenital disorder of glycosylation, type I (#614921)	Dilated cardiomyopathy (HP:0001644) Restrictive cardiomyopathy (HP:0001723) Left ventricular dilatation (HP:4000141) Left ventricular hypertrophy (HP:0001712) Heart failure (HP:0001635) Cardiomegaly (HP:0001640) Aortic coarctation (HP:0001680) Ventricular septal defect (HP:0001629) Arrhythmia (HP:0011675) Atrial fibrillation (HP:0005110) Bifid uvula (HP:0000193) Cleft palate (HP:0000175) Micrognathia (HP:0000347) Pierre-Robin sequence (HP:0000201) Macrocephaly (HP:0000256) Developmental delay (HP:0012758) Speech delay (HP:0000750) Intellectual disability (HP:0001249) Hypotonia (HP:0001252) Hepatopathy (HP:0001410) Short stature (HP:0004322) Exercise intolerance (HP:0003546) Biological and endocrinal abnormalities (HP:0031073)

Abbreviations: COP, continuation of pregnancy; F, female fetus; GA, gestational age; M, male fetus; TOP, termination of pregnancy; WG, weeks of gestation.

by the potential challenges in establishing the association between relevant variants and fetal phenotypes.

Uncommon and unreported phenotypes: from challenges in associating phenotype and genotype to expanding the clinical spectrum.

In 32.1% of cases, the identified prenatal features were uncommon or previously unreported in relation to known human disease genes. In these instances, establishing the association of pathogenic or likely pathogenic variants with an unexpected fetal phenotype proved to be challenging.

The additional features identified in our patients suggest the need to broaden the clinical spectrum of genetic disorders with prenatal onset. Our findings highlight the major clinical heterogeneity of prenatal genetic disorders. Before phenotype extension, a double diagnosis was systematically considered seeing as in previous postnatal cohorts of rare diseases, ES identified a double diagnosis in around 2%–3% of patients.²³ A double diagnosis can explain the phenotypic heterogeneity of genetic disorders with atypical or severe phenotypes in some cases. For example, the 22q11.2 deletion was identified in a large-for-gestational-age fetus with enlarged kidneys after ruling out a CNV/SNV involved in syndromic overgrowth. Although our data suggested a phenotypic extension, similar observations, postnatal genome sequencing, or functional tests are needed for validation.

For some cases, the absence of clinical features typically considered as recurrent hallmarks of a genetic disorder suggests that there are limitations when applying postnatal diagnostic criteria in a prenatal setting.

In addition, we reported for the first time prenatal features related to two monogenic disorders with well-documented postnatal data. We reported an early onset of LINS1-related disorder (increased nuchal translucency, IUGR and asymmetric cerebral ventricles), originally associated with neurodevelopmental, autistic, and psychiatric disorders (schizophrenia or anxiety). For the second fetus, neonatal features of PGM1-CDG have previously been reported. The severity of congenital malformation, including Pierre-Robin sequence and airway compression, may be associated with the residual PGM1 enzyme activity.

The examination of the fetus during pregnancy goes beyond the detection of structural defects. It is crucial for evaluating fetal growth and overall well-being, with particular attention to fetal movements and the adnexa. In the field of genome-wide genomic tests, gestational age is a major challenge in fetal medicine. Prenatal phenotype is dependent on gestational age, and a fetus' gestational age influences the detection and accurate description of abnormalities. In early pregnancy, pES analysis may be challenging with limited or unspecific phenotypes leading to inconclusive tests (negative or identification of VUS). In addition, early prenatal imaging may limit the association between phenotype and genotype, especially in case of multiple diagnosis, as illustrated by our patient carrying the ASCC1 and CSPP1 variants. Conversely, in late pregnancy, an exhaustive phenotype combining all fetal findings provided from prenatal follow-up helps with pES interpretation. However, some parts of the fetus become

technically difficult to examine. Although there is no gestational age limit for genetic testing during pregnancy, a late prescription may compromise the possibility of providing a result within the pregnancy timeframe. In addition, there are delays in providing information to the parents and the personalization of perinatal management (continuation or termination of pregnancy, tailored therapy...) are delayed.

In inconclusive cases, prenatal follow-up is needed to obtain additional insight and findings, especially in case of VUS, for which particular examinations may be offered. Following the initial analysis, reanalysis of inconclusive cases should be performed using additional data gleaned from prenatal follow-up. This interactive approach involving clinico-biological consultations should be conducted within an MDT specialized in fetal genomic medicine. Prenatal phenotyping requires a high level of expertise in fetal imaging, for both initial assessments and follow-up with targeted examinations in case of VUS. The implementation of pES requires a network of experts at each step of the process: prenatal imaging, clinical genetics, and molecular biology.

4.2 | Perspectives

In the absence of prenatal phenotype-genotype correlation databases, identifying relevant variants may require the combination of several *in silico* tools. However, they have limitations, particularly for missense variants for which the damage prediction is discordant, or recessive disorders with pathogenic/likely pathogenic variants having allele frequencies >1%. Nevertheless, in a blinded reanalysis study, our team obtained complete concordance between trio-pES and postmortem ES for fetuses with multisystem malformations, suggesting the effectiveness of the genotype-first strategy.²⁴ However, there are no data so far in cases of isolated or minor congenital defects. In this context, longitudinal follow-up of inconclusive pES is needed to assess the added value of postnatal/postmortem features in reanalysis and to identify limitations related to prenatal phenotype and complete phenotype-genotype correlation databases.

Prenatal features have long been collected retrospectively following a postnatal diagnosis, thus compromising the accuracy of prenatal phenotyping. The implementation of pES in clinical practice offers new perspectives for describing fetal phenotypes and the natural history of genetic disorders with prenatal onset. Combining specific gene or condition series with unselected fetal cohorts may be useful for improving our understanding of fetal conditions. A large-scale compilation of the prenatal features associated with genetic disorders could enhance pES analysis. Integrating prenatal phenotypes into genotype-phenotype databases may increase the diagnostic yield of pES, reduce the rate of VUS, and reconsider fetal autopsy following conclusive pES.

Beyond a standardized and detailed prenatal examination, prenatal features must be uniformly described. Human Phenotype Ontology (HPO), a specific ontology for congenital disorders, is now widely used to describe postnatal phenotypical abnormalities.²⁵ Unfortunately, only a minority of HPO terms can be used in the prenatal

context. Moreover, some specific prenatal features have not yet been associated with an HPO term, that is, asymmetric cerebral ventricles without ventriculomegaly. Recently, the HPO consortium added 95 new terms specific to abnormal prenatal development.²⁶

In conclusion, our study provides novel data on the prenatal phenotypes of several different genetic disorders. Hopefully, collaborative compilation of prenatal data from pES cohorts will lead to a better understanding of fetal genetic disorders and facilitate their identification.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All fetuses were initially included in this AnDDI-prenatome study (NCT03964441), approved by the institutional review board and ethics committee (Comité de Protection des Personnes (CPP)). Written informed consent was obtained from all subjects and participating family members.

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