BRIEF COMMUNICATION

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Same performance of exome sequencing before and after fetal autopsy for congenital abnormalities: toward a paradigm shift in prenatal diagnosis?

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Prenatal exome sequencing could be complex because of limited phenotypical data compared to postnatal/portmortem phenotype in fetuses affected by multiple congenital abnormalities (MCA). Here, we investigated limits of prenatal phenotype for ES interpretation thanks to a blindly reanalysis of postmortem ES data using prenatal data only in fetuses affected by MCA and harboring a (likely)pathogenic variant or a variant of unknown significance (VUS). Prenatal ES identified all causative variant previously reported by postmortem ES (22/24 (92%) and 2/24 (8%) using solo-ES and trio-ES respectively). Prenatal ES identified 5 VUS (in four fetuses). Two of them have been previously reported by postmortem ES. Prenatal ES were negative for four fetuses for which a VUS were diagnosed after autopsy. Our study suggests that prenatal phenotype is not a limitation for implementing pES in the prenatal assessment of unsolved MCA to personalize fetal medicine and could influence indication of postmortem examination.

European Journal of Human Genetics (2022) 30:967–975; https://doi.org/10.1038/s41431-022-01117-7

INTRODUCTION

Congenital abnormalities (CA) occur in 2–5% of pregnancies and are the main cause of perinatal death (20–25%) [1]. Detecting CA, especially to identify genetic disorders that affect fetal prognosis, is one of the biggest challenges in prenatal care [2]. Current prenatal genetic assessment of fetal malformations based on standard karyotype and chromosomal micro-array analysis (CMA) identifies chromosomal abnormalities and pathogenic copy number variants (CNV) in approximately 20% and 6% of cases, respectively [3]. Identification of the causal genetic abnormalities is required for diagnosis; to adapt prenatal/perinatal management according to the prognosis; and to provide genetic counseling for the current pregnancy, and any subsequent pregnancies [4]. In negative cases (about 70%), the management is limited due to remaining challenges relative to genetic counseling [5].

In the last decade, several studies have highlighted the contribution of next-generation sequencing, particularly exome sequencing (ES), in the investigation of postnatally suspected monogenic diseases, with a mean diagnostic yield of approximately 40% [6]. The use of ES to investigate CA was progressively extended from postmortem to prenatal diagnosis. It has been illustrated that the diagnostic yield seems to be lower than for other postnatal indications (i.e intellectual disability), with an overall diagnostic yield of approximately 20%. However, the diagnostic value of pES in CA should no longer be controversial, with a diagnostic yield greater than the additive value of CMA relative to karyotype [3]. However, a high variation has been

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Received: 13 December 2021 Revised: 5 March 2022 Accepted: 19 April 2022
Published online: 16 May 2022

reported (from 2 to 71%) depending on the series' inclusion criteria and the methodology used [4, 7, 8].

Although the diagnostic yield of prenatal ES (pES) appears to be lower, running prenatal genetic tests allows parents to obtain additional information about the disease and prognosis, and may influence pregnancy outcome and future family planning [9]. Implementation of pES in clinical practice could be limited by cost, turnaround time, interpretation based on a "partial" fetal phenotype [4], incidental findings, and management of variants of unknown significance (VUS).

In case of lethal CA, postmortem phenotype, mostly report in clinical databases, with potential additional information provided by prenatal imaging, is considered as the reference for genetic investigations. It could be reconsidered by improvement of prenatal imaging, parental reluctance for postmortem examination and development of genomic medicine.

To investigate limits of prenatal phenotype for ES interpretation, we compared results of ES analyzed using prenatal data only versus postmortem clinical data.

MATERIAL AND METHODS

Affected cases

We performed an ancillary study from the collaborative French cohort including 95 fetuses with lethal and unsolved multisystemic CA investigated by postmortem solo-ES (NCT02512354-FOETEX) [10]. Briefly, fetuses included after termination of pregnancy (TOP), intrauterine or neonatal death, had at least two congenital malformations and no etiological clinical diagnosis after fetal examinations and current investigations (CMA, targeted sequencing). For the present study, we included all fetuses with a likely pathogenic or a pathogenic (LP/P) variant or a VUS identified by postmortem solo-ES. In the FOETEX study, clinical data available for the ES analysis were almost exclusively from the postmortem examination, the family history and the risk factors (consanguinity, previous TOP, intrauterine fetal death…); prenatal data were not detailed. Parents provided written informed consent and the study was approved by the local ethics committee.

Prenatal clinical data and ES analysis

We retrospectively collected all prenatal data in a standardized phenotyping report, especially fetal imaging reports (ultrasound, CT-scan, MRI), biological investigations (Down syndrome screening, tests performed on chorionic villus sampling or amniotic fluid), risk factors (toxic or work-related exposure…) and obstetrical diseases. Prenatal data were compiled in a standardized report by a specialist in fetal imaging without knowledge of the pregnancy outcome.

ES data were blindly reanalyzed by biologists that did not have previously participated in the initial ES analysis based on fetal autopsy. pES analysis was performed using a sequential approach: first, a solo-pES analysis to limit costs, using the multistep methodology previously described by Lefebvre et al., and second a trio-ES (trio-pES) for negative cases. To compare pES and postmortem ES, the analysis of pES data was made thanks to the pipeline previously used for the original study (Lefebvre et al.,) without updating of annotation and databases. Candidate variants were multidisciplinary discussed and classified following the American College of Medical Genetics and Genomics recommendations [11]. Secondary and incidental findings were not allowed.

RESULTS

A total of 32 fetuses (17 males, 15 females) were included, involving 24 cases with a causal diagnosis and eight cases harboring a VUS after postmortem solo-ES. Clinical data are summarized in Table 1.

Solo-pES identified a LP/P variant in 22 fetuses, all of them previously identified by postmortem ES (Table 1).

Subsequent trio-pES identified the causal diagnoses for the two last cases solved by postmortem ES (F.10 and F.17), increasing the diagnostic yield from 92% (22/24) to 100%, and 5 VUS in 4 fetuses. For F.25 with a phenotype suggestive of a congenital bone disorder, a VUS in *INPPL1* and *COL1A1* was respectively identified by prenatal and postmortem ES. For two cases with a syndromic congenital diaphragmatic hernia, pES identified a VUS in *LRP2*, not reported by postmortem ES, and associated for one case with a *RLIM* variant (previously diagnosed). Discrepancies between prenatal and postmortem VUS are summarized in Table 2. Following our stepwise strategy, pES was negative for four fetuses with VUS identified by postmortem ES (Fig. 1).

DISCUSSION

We report the first pilot study comparing the performance of ES for multisystemic CA before and after fetal autopsy.

In our series, trio-pES was completely concordant with postmortem ES for LP/P variant, either typical or atypical phenotype. Considering variability of fetal phenotype, trio-pES should be systematically favored. However, when parental DNA is not available, solo-pES could be an alternative with a highly concordance with postmortem ES (92%). Our data suggest that the prenatal phenotype, although limited, is not a limitation for interpretation of pES in case of multisystemic CA.

The implementation of pES in standard care would change the practice of prenatal diagnosis and require a highly specialized multidisciplinary team for prenatal imaging, interpretation of genetic variants and validation by phenotype-genotype correlation. During pregnancy, reverse-phenotyping using additional imaging or biological tests could be necessary to corroborate diagnosis in case of a LP/P variant or confirm, or not, causality of VUS, one major challenge of pES (4 to 32%) [12, 13]. Discrepancy between VUS considered by prenatal and postmortem analysis could be explained by poor phenotype-genotype correlations, limited in-silico prediction scores for selected variants, and clinical features included in nonspecific phenotype. Contrary to personalized postnatal medicine, there are no guidelines for reporting VUS from pES [11]. The uncertainty and limitations in prenatal counseling, and the large spectrum of adverse consequences must be considered carefully [9]. Therefore, we suggest to only considered VUS in OMIM genes with a high potential for clinical significance and if complementary tests are available to reclassify variant. Finally, for negative pES, reanalysis of ES using additional data provided by longitudinal pregnancy follow-up could lead to a diagnosis that was initially not considered. Currently, postnatal and postmortem clinical data of genetic disorders are mainly reported in clinical databases. The lack of specific database reporting prenatal data limits the development of the genomic prenatal medicine. It is now essential to develop them.

Implementation of pES and increase of prenatal data may influence indications of postmortem examinations. While fetal autopsy provides additional features in around 70% of cases, but it influences in only 25% of cases. In a large majority of cases, added value of fetal autopsy is limited with a total agreement with prenatal imaging or minor additional features in 45% and 30% of procedures respectively [14]. The implementation of whole-genome analyses should encourage us to reconsider the additive value of fetal autopsy. This is consistent with our results that show similar efficiency of trio-pES and postmortem ES. When pES identify LP/P variant in typical phenotypes, autopsy could be reconsidered because of no added value for genetic counseling. Conversely, postmortem examination remains essential in negative pES, VUS or atypical ultrasound phenotype secondary to a LP/P variant to look for additional phenotypic arguments and/or perform complementary etiological investigations on fetal tissues different from amniotic or fetal blood. New approach of fetal autopsy, based on virtual autopsy with or not or minimal invasive sampling, could be considered according to the prenatal phenotype [15]. These options should reduce parent's reluctance and improve genetic counseling.

Previously offered after the pregnancy, implementation of pES may have a major clinical utility for patients and medical teams. Without diagnostic failure, our data suggest that offering

Table 1. Causal variants identified by the multistep strategy: prenatal and postnatal clinical and molecular data.

	Data from FOETEX study (NCT02512354)							Data from the present ancillary study				
Cases	**Sex**	**Consan-guinity**	**Family history of MCA**	**Pregnancy outcome**	**Postmortem phenotype**	**Postmortem solo ES results Gene (OMIM number) Variation cDNA and protein Inheritance**	**Related phenotype (OMIM number)**	**GA of first US abnormalities (WG)**	**Prenatal phenotype (US ± MRI)**	**Prenatal investigations[a]**	**Prenatal solo ES results**	**Prenatal trio ES results**
F.1	M	No	No	TOP 26 WG	Distal arthogryposis, unilateral preaxial hexadactyly	*MYH3 (160720)* NM_002470.4: c.2015G > A p.(Arg672His) Sporadic (AD)	Arthrogryposis distal, type 2 A (193700) type 2B (601680) type 8 (178110)	24	Dysmorphism, retrognathia, bilateral talipes, clenched hands	NP	+[a]	NP
F.2	F	No	No	TOP 35 WG	Ascites, hepatic fibrosis, splenomegaly	*NPC1 (607623)* NM_000271.5: c.2819 C > T p.(Ser940Leu) Homozygous (AR)	Niemann-Pick disease, type C1 or type D (257220)	23	Ascites, hyperechoic bowel, hepatosplenomegaly, hydrops fetalis, hypokinetic cardiomyopathy	Parental screening for cystic fibrosis: negative PCR on AF for TORCH infection: negative	+[a]	NP
F.3	M	No	TOP 22WG for bilateral VMG, hyperechoic kidneys	TOP 35 WG	IUGR, posterior corpus callosum hypoplasia, bilateral labial and cleft palate, bilateral microcoria	*B3GALTL (610308)* NM_194318.4:c.660 + 1 G > A p.? Homozygous (AR)	Peters-plus syndrome (261540)	22	IUGR, posterior corpus callosum hypoplasia, bilateral labial and cleft palate	NP	+[a]	NP
F.4	M	No	No	IUFD 30 WG	Severe IUGR, short neck, facial dysmorphism, right labial cleft and cleft palate	*TFAP2A (107580)* NM_001032280. 3: c.688 C > T p.(Arg230Trp) Paternally inherited (AD)	Branchiooculofacial syndrome (113620)	23	IUGR, unilateral labial and cleft palate, retrognathia, craniostenosis	NP	+[a]	NP
F.5	F	No	No	TOP 26 WG	IUGR, hydramnios, microcrania, retrognathia, vermian hypoplasia, auricular external canal atresia, broad hands, syndactyly, interauricular communication	*SLC25A24 (608744)* NM_013386.4:c.649 C > T p.(Arg217Cys) Sporadic (AD)	Fontaine progeroid syndrome (612289)	23	IUGR, polyhydramnios, 2-3 syndactyly, atrioventricular canal defect	NP	+[a]	NP
F.6	F	Yes	No	TOP 34 WG	IUGR, microcephaly, corpus callosum hypoplasia, optic nerve coloboma, hemivertebrae	*STAG2 (300826)* NM_001042749.2: c.2857 C > T p.(Arg953[a]) Sporadic (XL)	X-linked neurodevelopmental disorder with craniofacial abnormalities (NEDXCF) (301022)	21	IUGR, microcephaly, corpus callosum hypoplasia, interhemispheric cyst, delayed gyration, cerebellum hypoplasia, intestinal malrotation	PCR on AF for TORCH infection: negative	+[a]	NP
F.7	M	No	TOP 21 WG (occipital encephalocele, hexadactyly, polysplenia). Negative ciliopathy panel sequencing	TOP 30 WG	IUGR, ventricular septal defect, craniostenosis, horseshoe kidney	*DPH1 (603527)* NM_001383.4:c.374 T > C p.(Leu125Pro) Homozygous (AR)	Developmental delay with short stature, dysmorphic features and sparse hair (616901)	23	IUGR, complex congenital heart disease (transposition of the main arteries, atrioventricular canal defect, interventricular septal defect), craniostenosis, dysmorphism, curved penis	NP	+[a]	NP
F.8	M	No	No	TOP 29 WG	Cutis laxa, CCA, mineralization delay	*ALDH18A1 (138250)* NM_002860.4: c.1273 C > T p.(Arg425Cys) NM_002860.4: c.177del p.(Lys59Asnfs[a]9) Compound heterozygous (AR)	Cutis laxa, autosomal recessive, type IIIA (219150)	23	Severe IUGR, corpus callosum agenesia, lissencephaly, cerebellum hypoplasia, short long bones	PCR on AF for TORCH infection: negative	+[a]	NP
F.9	F	No	No	TOP 30 WG	IUGR, corpus callosum hypoplasia, ventriculomegaly, low-set ears, narrow mouth	*ARX (300382)* NM_139058.2: c.1374_1383del p.(Pro459[a]) Sporadic (XL)	Hydranencephaly with abnormal genitalia, Lissencephaly, X-linked 2 (300215)	22	IUGR, bilateral ventriculomegaly, corpus callosum agenesia	PCR on AF for TORCH infection: negative Screening of maternal coagulation disorders: negative	+[a]	NP

Table 1. continued

Cases	Data from FOETEX study (NCT02512354)							Data from the present ancillary study				
	Sex	Consan-guinity	Family history of MCA	Pregnancy outcome	Postmortem phenotype	Postmortem solo ES results Gene (OMIM number) Variation cDNA and protein Inheritance	Related phenotype (OMIM number)	GA of first US abnormalities (WG)	Prenatal phenotype (US ± MRI)	Prenatal investigations[a]	Prenatal solo ES results	Prenatal trio ES results
F.10	F	No	No	TOP 26 WG	IUGR, hygroma, facial dysmorphism, right retinal coloboma, syndactyly, broad hallux, left congenital diaphragmatic hernia, toe nail hypoplasia	*PORCN (300651)* NM_203475.3: c.1094 G > A p.(Arg365Gln) Sporadic (XL)	Focal dermal hypoplasia (305600)	21	Severe IUGR, left congenital diaphragmatic hernia (severe lung hypoplasia)	NP	–	+[a,b]
F.11	M	No	TOP for 47, XX, + 21	TOP 17 WG	Hydrops, interventricular communication, brachydactyly, partal vermian hypoplasia	*HRAS (190020)* NM_005343.4:c.38 G > A p.(Gly13Asp) Sporadic (AD)	Congenital myopathy with excess of muscle spindles (218040) Costello syndrome (218040)	12	Cystic hygroma (5 mm), hydrops fetalis, talipes, cleft palate	NP	+[a]	NP
F.12	M	No	No	TOP 33 WG	IUGR, CCA	*SMARCE1 (603111)* NM_003079.5:c.276 G > C p.(Lys92Asn) Sporadic (AD)	Coffin-Siris syndrome 5 (616938)	24	IUGR, microcephaly, delayed gyration, corpus callosum agenesia, complex congenital heart disease (heterotaxy, left superior vena cava, interventricular septal defect, ductus venosus agenesia)	PCR on AF for TORCH infection: negative	+[a]	NP
F.13	F	No	No	TOP 26 WG	Hydrocephaly, aqueductal stenosis, camptodactyly, unilateral simian crease	*SMARCC1 (601732)* NM_003074.3: c.2782–1 G p.? Sporadic (AD)	NA[§]	23	Hydrocephaly probably secondary to aqueductal stenosis	NP	+[a]	NP
F.14	M	No	No	TOP 26 WG	Hydrops, retrognathia, posterior cleft palate	*HRAS (190020)* NM_005343.4:c.38 G > A p.(Gly13Val) Sporadic (AD)	Congenital myopathy with excess muscle spindles (218040) Costello syndrome (218040)	11	Cystic hygroma, hydrops fetalis, partial cerebellum agenesia, hepatomegaly	PCR on AF for TORCH infection: negative Biochemical screening for inherited metabolic disorders on AF: negative	+[a]	NP
F.15	M	No	No	ND	Hypertelorism, bilateral clubfeet, lung hypoplasia	*MUSK (601296)* NM_005592.4:c.308 A > G p.(Asn103Ser) NM_005592.4: c.2357 G > A p.(Trp786[a]) Compound heterozygous (AR)	Fetal akinesia deformation sequence (208150) Myasthenic syndrome, congenital, 9, associated with acetylcholine receptor deficiency (616325)	30	Polyhydramnios, right congenital diaphragmatic hernia (moderate lung hypoplasia), bilateral talipes	NP	+[a]	NP
F.16	M	No	No	TOP 27 WG	Facial dysmorphism, hypoplastic left heart, left kidney hypoplasia, cleft palate	*KMT2D (602113)* NM_003482.3: c.13884del p.(Thr4629Profs[a]11) Sporadic (AD)	Kabuki syndrome 1 (147920)	23	Bilateral pleural effusion, hypoplastic left heart	NP	+[a]	NP
F.17	F	No	ND after C-section for abnormal fetal heart rate. Severe hydrocephaly	IUFD 28 WG	Hydrops, IUGR, cerebral calcification, hepatomegaly	*TREX1 (606609)* NM_033629.6: c.397del p.(Leu133Cysfs[a]27) Homozygous (AR)	Aicardi-Goutieres syndrome 1, dominant and recessive (225750)	22	Hyperechoic bowel, non-visualization of gallbladder, hydrops fetalis, dolichocephaly, abnormal corpus callosum, cardiomegaly	Parental screening for cystic fibrosis: negative PCR on AF for TORCH infection: negative	–	+[a,c]
F.18	M	No	No	TOP 19 WG	IUGR, microcephaly, median labial and palate cleft, arthrogryposis, gyration defect, cerebellar hypoplasia, hypocampus hypoplasia	*TUBA1A (602529)* NM_006009.4:c.959 G > A p.(Arg320His) Sporadic (AD)	Lissencephaly 3 (611603)	16	Severe IUGR, arthrogryposis, dysmorphism, abnormal posterior cranial fossa, subcutaneous edema	NP	+[a]	NP

Table 1. continued

	Data from FOETEX study (NCT02512354)							Data from the present ancillary study				
Cases	**Sex**	**Consan-guinity**	**Family history of MCA**	**Pregnancy outcome**	**Postmortem phenotype**	**Postmortem solo ES results Gene (OMIM number) Variation cDNA and protein Inheritance**	**Related phenotype (OMIM number)**	**GA of first US abnormalities (WG)**	**Prenatal phenotype (US ± MRI)**	**Prenatal investigations[a]**	**Prenatal solo ES results**	**Prenatal trio ES results**
F.19	M	No	No	IUFD 28 WG	IUGR, ichthyosis, intervillitis	*ERCC2 (126340)* NM_000400.3: c.2164 C > T p.(Arg722Trp) NM_000400.3: c.2092 C > T p.(Gln698[a]) Compound heterozygous (AR)	Trichothiodystrophy 1, photosensitive (601675)	22	Severe IUGR, hyperechoic bowel, oligohydramnios, umbilical cord cyst	NP	+[a]	NP
F.20	M	No	No	ND	Facial dysmorphism, hypoplastic third phalanges, left diaphragmatic hernia, complex cardiopathy, hypospadias	*NIPBL (608667)* NM_133433.4: c.3425dup p.(Gly1143Trpfs[a]13) Sporadic (AD)	Cornelia de Lange syndrome 1 (122470)	12	Cystic hygroma, IUGR, right congenital diaphragmatic hernia (severe lung hypoplasia), tetralogy of Fallot, coloboma, polyhydramnios	Biochemical screening for Smith Lemli Opitz syndrome: negative	+[a]	NP
F.21	F	No	No	TOP 24 WG	IUGR, craniostenosis, facial dysmorphism, cervical and thoracic hemivertebrae, bilateral ventriculomegaly, bifid uterus	*FGFR2 (176943)* NM_000141.4: c.1052 C > G p.(Ser351Cys) Sporadic (AD)	Apert syndrome (101200) Crouzon syndrome (123500) Pfeiffer syndrome (101600) Saethre-Chotzen syndrome (101400)	22	Craniostenosis, thoracic hemivertebrae, bilateral ventriculomegaly	NP	+[a]	NP
F.22	M	No	No	TOP 33 WG	Potter's sequence, proximal tubule agenesis, poor biliary ducts, renal hypoplasia	*ACE (106180* NM_000789.4: c.3503 + 1 G > C p.? Paternal isodisomy (AR)	Renal tubular dysgenesis (267430)	31	IUGR, oligohydramnios, bilateral CAKUT	Fetal beta-2-microglobulin: severe renal damage	+[a]	NP
F.23	M	No	No	TOP 23 WG	Hygroma, Severe hydrocephaly, CCA, heterotaxia, renal dysplasia, short femur, common mesentery	*ARID1A (603024)* NM_006015.6: c.4860dup p.(Pro1621Tyrfs[a]27) Sporadic (AD)	Coffin-Siris syndrome 2 (614607)	21	IUGR, short long bones, severe bilateral hydrocephaly	NP	+[a]	NP
F.24	F	No	No	TOP 20 WG	Severe micromelic nanism, CCA, right hydronephrosis	*COL2A1 (120140)* NM_001844.5: c.3329 G > T p.(Gly1110Val) Sporadic (AD)	Achondrogenesis, type II or hypochondrogenesis (200610)	13	Cystic hygroma, skeletal dysplasia with severe micromelia, bilateral talipes and hypoplastic thorax	NP	+[a]	NP

M male fetus, *F* female fetus, *TOP* termination of pregnancy, *ND* neonatal death, *IUFD* intrauterine fetal death, *WG* weeks of gestation, *IUGR* intrauterine growth retardation, *CAKUT* Congenital Anomalies of the Kidneys and Urinary Tracts, *NP* not performed, *PCR* polymerase chain reaction, *AF* amniotic fluid, *CCA* corpus callosum agenesis, *AD* autosomal dominant inheritance, *AR* autosomal recessive inheritance, *XL* X-linked inheritance.

[a]similar to the postmortem solo ES results.

[b]variant initially not considered among missense variant and required family segregation to identify de novo status.

[c]variant initially not considered because of extreme phenotype suggestive of a congenital infection, not described in prenatal cases of AGS syndrome, report by our team (Bourgon, N. et al. Prenatal presentation of Aicardi-Goutières syndrome: Nonspecific phenotype necessitates exome sequencing for definitive diagnosis. Prenat Diagn 39, 806–810 (2019)). § SMARCC1 is not an OMIM gene but was reported in two publications (Furey, C. G. et al. De Novo Mutation in Genes Regulating Neural Stem Cell Fate in Human Congenital Hydrocephalus. Neuron 99, 302-314.e4 (2018) and Jin, S. C. et al. Exome sequencing implicates genetic disruption of prenatal neuro-gliogenesis in sporadic congenital hydrocephalus. Nat Med 26, 1754–1765 (2020)).

Table 2. Variants of unknown significance identified in OMIM causing disease genes: prenatal and postnatal clinical and molecular data.

	Data from FOETEX study (NCT02512354)							Data from the present ancillary study					
Cases	**Sex**	**Consan-guinity**	**Family history of MCA**	**Pregnancy outcome**	**Postmortem phenotype**	**Postmortem solo ES results Gene (OMIM number) Variation cDNA and protein Inheritance**	**Related phenotype (OMIM number)**	**GA of first US abnormalities (WG)**	**Prenatal phenotype (US ± MRI)**	**Prenatal trio ES results**	**Postmortem trio ES results Gene (OMIM number) Variation cDNA and protein Inheritance**	**Related phenotype (OMIM number)**	**Reason of discrepancy**
F.30	F	No	No	TOP 25 WG	IUGR, craniostenosis, holoprosencephaly, CCA, gyration defect, hemihypertrophia	*MED23* (605043) NM_004830.4: c.1033 C > T/ p.(Gln345[a]) NM_004830.4: c.1832G > A/ p.(Arg611Gln) Compound heterozygous (AR)	Mental retardation, autosomal recessive 18 (614249)	22	IUGR, holoprosencephaly	VUS	*MED23* (605043) NM_004830.4: c.1033 C > T/ p.(Gln345[a]) NM_004830.4: c.1832G > A/ p.(Arg611Gln) Compound heterozygous (AR)	Mental retardation, autosomal recessive 18 (614249)	NA
F.31	M	No	No	TOP 34 WG	IUGR, bilateral diaphragmatic hernia, hypospadias, facial dysmorphism	*RLIM* (300379) NM_016120.4: c.170 G > T/ p.(Gly57Val) Maternally inherited (XL)	Tonne-Kalscheuer syndrome (300978)	23	IUGR, diaphragmatic hernia	VUS VUS	*RLIM* (300379) NM_016120.4: c.170 G > T/ p.(Gly57Val) Maternally inherited (XL)	Tonne-Kalscheuer syndrome (300978)	NA
											LRP2 (600073) NM_004525.2: c.6160 G > A/ p.(Asp2054Asn) Recessive (AR)	Donnai-Barrow syndrome (222448)	*LRP2* variants are involved in syndromic congenital diaphragmatic hernia[a]
F.25	F	No	No	TOP 28 WG	Facial dysmorphism, shortening of the long bones, narrow thorax	*COL1A1 (120150)* NM_000088.3: c.2831 G > T/ p.(Gly944Val) Sporadic (AD)	Caffey disease (114000)	24	IUGR, shortened long bones	VUS	*INPPL1* (258480) NM_001567.3: c.2535 C > G/ p.Ile845Met Recessive (AR)	Opsismodysplasia (258480)	*COL1A1* variant was considered by postmortem ES because of sporadic occurrence. This variant is absent from databases. Poor phenotype-genotype correlation: no fracture, no hypomineralisation (*COL1A1*).
F.26	F	No	No	TOP 22 WG	Asymmetrical microphtalmia, cleft palate, maxillar hypoplasia, arhinencephaly, CCA, unilateral semicircular canal hypoplasia, right diaphragmatic hernia	*COL7A1* (120120) NM_000094.3: c.1613G > A/ p.(Arg538His) NM_000094.3: c.4585 C > T/ p.(Arg1529Cys) Compound heterozygous (AR)	Epidermolysis bullosa dystrophica (EBD) inversa (226600) EBD, Bart type (132000) Epidermolysis bullosa dystrophica, AD (131750) Epidermolysis bullosa dystrophica, AR (226600) Epidermolysis bullosa pruriginosa (604129) Epidermolysis bullosa, pretibial (131850) Toenail dystrophy, isolated (607523) Transient bullous of the newborn (131705)	21	CCA, diaphragmatic hernia	VUS	*LRP2* (600073) NM_004525.2: c.2603 C > G/ p.Thr868Ser Recessive (AR)	Donnai-Barrow syndrome (222448)	*LRP2* variants are involved in syndromic congenital diaphragmatic hernia[a] No phenotype-genotype correlation (*CO71A1*).
F.27	F	No	No	TOP 28 WG	IUGR, diaphragmatic hernia, facial dysmorphism, superior short oral frenula, bilateral clino/camptodactyly fingers II and V, bilateral simian crease, diaphragmatic hernia, gallbladder agenesis, four accessory spleens, short corpus callosum	*CREBBP* (600140) NM_001079846.1: c.5470 G > C/ p.(Ala1824Pro) Sporadic (AD)	Rubinstein-Taybi syndrome 1 (180849)	22	IUGR, diaphragmatic hernia, bilateral pyelectasis, corpus callosum hypoplasia	–	Negative	–	*CREBBP* variant was considered by postmortem ES because of sporadic occurrence. Other causal missense variants have previously been reported in the same domain. Postmortem phenotype is extreme and prenatal phenotype limited.

Table 2. continued

Cases	Data from FOETEX study (NCT02512354)							Data from the present ancillary study					
	Sex	Consan-guinity	Family history of MCA	Pregnancy outcome	Postmortem phenotype	Postmortem solo ES results Gene (OMIM number) Variation cDNA and protein Inheritance	Related phenotype (OMIM number)	GA of first US abnormalities (WG)	Prenatal phenotype (US ± MRI)	Prenatal trio ES results	Postmortem trio ES results Gene (OMIM number) Variation cDNA and protein Inheritance	Related phenotype (OMIM number)	Reason of discrepancy
F.28	M	No	No	TOP 14 WG	Tracheal atresia, anal atresia, uretral atresia, bilateral renal dysplasia	*FRAS1* (607830) NM_025074.7: c.5924 T > C/ p.(Ile1975Thr) Paternally inherited (AR)	Fraser syndrome 1 (219000)	12	Megacystis	–	Negative	–	*FRAS1* variant considered after autopsy because of tracheal atresia. However, in-silico prediction scores were border, and another hit was not identified.
F.29	F	No	No	TOP 15 WG	Hydrops, multiple pterygium	*HYDIN* (610812) NM_001270974.2: c.3881 C > G/ p.(Ser1294Cys) NM_001270974.2: c.3365 T > C/ p.(Leu1122Pro) Compound heterozygous (AR)	Ciliary dyskinesia, primary, 5 (608647)	13	Fetal akinesia	–	Negative	–	Compound heterozygous *HYDIN* variante were not considered by prenatal reanalysis because of a low VAF (20 and 22%) and absence of phenotype-genotype correlation.
F.32	M	No	No	TOP 31 WG	IUGR, bifid penis	*SOS2* (601247) NM_006939.4: c.2249 T > C/ p.(Ile750Thr) Paternally inherited (AD)	Noonan syndrome 9 (616559)	23	IUGR, disorder of sexual development	–	Negative	–	*SOS2* variant, inherited from healthy father, absent from databases and in silico predict deleteriousness. Noonan syndrome is characterized by clinical variability and incomplete penetrance. Poor phenotype-genotype correlation.

M male fetus, *F* female fetus, *TOP* termination of pregnancy, *ND* neonatal death, *IUFD* intrauterine fetal death, *WG* weeks of gestation, *IUGR* intrauterine growth retardation, *NP* not performed, *CCA* corpus callosum agenesis, *AD* autosomal dominant inheritance, *AR* autosomal recessive inheritance, *XL* X-linked inheritance.

[a]Wynn, J., Yu, L. & Chung, W. K. Genetic causes of congenital diaphragmatic hernia. Semin Fetal Neonatal Med 19, 324–330 (2014).

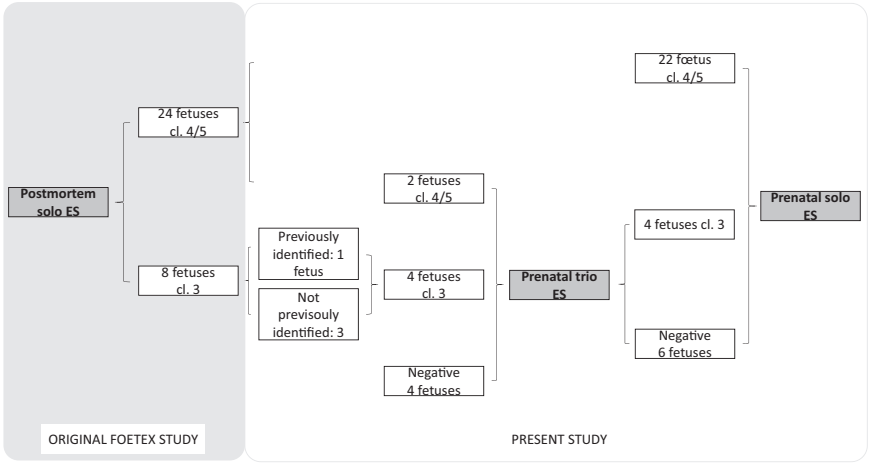

Fig. 1 Concordance between prenatal and postmortem ES. From left to right, variant identified by postmortem ES (original FOETEX study) and match with variants identified by prenatal reanalysis.

pES does not limit the genetic counseling. However, the limits of ES (identification of structural, noncoding/epigenetics variations or repeat expansions) must be considered according to the fetal phenotype and common related disorders [16]. According to fetal phenotype, the timing of genetics tests offered should consider parental wishes following the pre-test counseling. pES also improves the prenatal prognostic assessment and help parents in decision-making about pregnancy outcome (termination or continuation of the pregnancy). It is also a key factor for medical teams about acceptance of late TOP in some countries or customization of perinatal care: adjustment of monitoring, planning delivery, and neonatal care (resuscitation, specific medications, palliative care…) [17]. pES appears as a powerful tool for personalized fetal medicine [18].

In conclusion, pES could now be extensively implemented in clinical practice to investigate MCA with a similar effectiveness than postmortem ES. A trio strategy should be favored. This study also leads to reconsider the practices of fetal autopsy when pES identify a causal etiological diagnosis.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author (Pr Christel THAUVIN-ROBINET, mail: christel.thauvin@chu-dijon.fr) on reasonable request.

REFERENCES

1. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385:430–40.
2. Best KE, Rankin J, Dolk H, Loane M, Haeusler M, Nelen V, et al. Multilevel analyses of related public health indicators: the European Surveillance of Congenital Anomalies (EUROCAT) Public Health Indicators. Paediatr Perinat Epidemiol. 2020;34:122–9.
3. Hillman SC, McMullan DJ, Hall G, Togneri FS, James N, Maher EJ, et al. Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2013;41:610–20.
4. Normand EA, Braxton A, Nassef S, Ward PA, Vetrini F, He W, et al. Clinical exome sequencing for fetuses with ultrasound abnormalities and a suspected Mendelian disorder. Genome Med. 2018;10:74.
5. Harris S, Gilmore K, Hardisty E, Lyerly AD, Vora NL. Ethical and counseling challenges in prenatal exome sequencing. Prenat Diagn. 2018;38:897–903.
6. Dillon OJ, Lunke S, Stark Z, Yeung A, Thorne N, Melbourne Genomics Health A, et al. Exome sequencing has higher diagnostic yield compared to simulated disease-specific panels in children with suspected monogenic disorders. Eur J Hum Genet. 2018;26:644–51.
7. Lord J, McMullan DJ, Eberhardt RY, Rinck G, Hamilton SJ, Quinlan-Jones E, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet. 2019;393:747–57.
8. Pratt M, Garritty C, Thuku M, Esmaeilisaraji L, Hamel C, Hartley T, et al. Application of exome sequencing for prenatal diagnosis: a rapid scoping review. Genet Med. 2020;22:1925–34.
9. Wou K, Weitz T, McCormack C, Wynn J, Spiegel E, Giordano J, et al. Parental perceptions of prenatal whole exome sequencing (PPPWES) study. Prenat Diagn. 2018;38:801–11.
10. Lefebvre M, Bruel A-L, Tisserant E, Bourgon N, Duffourd Y, Collardeau-Frachon S, et al. Genotype-first in a cohort of 95 fetuses with multiple congenital abnormalities: when exome sequencing reveals unexpected fetal phenotype-genotype correlations. J Med Genet. 2021;58:400–13.
11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, 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;17:405–24.
12. Deden C, Neveling K, Zafeiropopoulou D, Gilissen C, Pfundt R, Rinne T, et al. Rapid whole exome sequencing in pregnancies to identify the underlying genetic cause in fetuses with congenital anomalies detected by ultrasound imaging. Prenat Diagn. 2020;40:972–83.
13. Petrovski S, Aggarwal V, Giordano JL, Stosic M, Wou K, Bier L, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet. 2019;393:758–67.
14. Godbole K, Bhide V, Nerune S, Kulkarni A, Moghe M, Kanade A. Role of fetal autopsy as a complementary tool to prenatal ultrasound. J Matern Fetal Neonatal Med. 2014;27:1688–92.
15. Cannie M, Votino C, Moerman P, Vanheste R, Segers V, Van Berkel K, et al. Acceptance, reliability and confidence of diagnosis of fetal and neonatal virtuopsy compared with conventional autopsy: a prospective study. Ultrasound Obstet Gynecol. 2012;39:659–65.
16. Burdick KJ, Cogan JD, Rives LC, Robertson AK, Koziura ME, Brokamp E, et al. Limitations of exome sequencing in detecting rare and undiagnosed diseases. Am J Med Genet A. 2020;182:1400–6.
17. Tolusso LK, Hazelton P, Wong B, Swarr DT. Beyond diagnostic yield: prenatal exome sequencing results in maternal, neonatal, and familial clinical management changes. Genet Med. 2021;23:909–17.
18. Bianchi DW. From prenatal genomic diagnosis to fetal personalized medicine: progress and challenges. Nat Med. 2012;18:1041–51.

ACKNOWLEDGEMENTS

The authors thank the parents for their participation. The authors also thank cnrgh for exome sequencing, the University of Burgundy Center de Calcul (ccuB) for providing technical support and management of the informatics platform, and the genematcher platform for data sharing.

AUTHOR CONTRIBUTIONS

Conceptualization: CT-R. Data curation: YD. Formal analysis: NB, ML, A-LB, PK, SN, CP, JT, FTM-T, SM, AS, AG, JD, AV. Funding acquisition: JT, CT-R, ML. Writing-original draft: NB. Writing-review and editing: CT-R.

FUNDING

This work was funded by the Program Hospitalier de Recherche Clinique (PHRC) Interregional 14-013, the Regional Council of Burgundy and the "Fonds Européen de Développement Régional" (FEDER). Several authors are members of the ERN ITHACA. This study was funded by interregional French Phrc interregional 14-013 FOeTeX.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All fetuses were initially included in this Foetex study, approved by our regional institutional review board and ethics committee (Comité de Protection des Personnes (CPP) EST I (Dijon)). Informed written consent was obtained from all subjects and participating family members.

ADDITIONAL INFORMATION

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