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Exome sequencing in fetuses with congenital diaphragmatic hernia in a nationwide cohort

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Abstract

Objective: To evaluate the diagnostic yield of exome sequencing (ES) in fetuses and neonates with prenatally detected congenital diaphragmatic hernia (CDH) and normal copy number variant (CNV) analysis.

Methods: We conducted a retrospective cohort study of prenatally diagnosed CDH cases seen between 2019 and 2022. All cases who underwent prenatal or postnatal genetic testing were reviewed. The results from the ES analysis that identified pathogenic or likely pathogenic single nucleotide variants are described.

Results: In total, 133 fetuses with CDH were seen, of whom 98 (74%) had an isolated CDH and 35 (26%) had a complex CDH (associated structural anomalies) on prenatal examination. ES was performed in 68 cases, and eight pathogenic or likely pathogenic variants were found, accounting for a 12% diagnostic yield (10% [5/50] in isolated cases and 17% [3/18] in complex CDH).

Conclusions: In 12% of fetuses and neonates with CDH and normal CNV analysis results, pathogenic or likely pathogenic variants were identified with ES. These data indicate that there is a substantial diagnostic yield when offering ES in prenatally detected CDH, both in complex and isolated cases.

Key points

What's already known about this topic?

- More than 100 syndromes are reportedly associated with congenital diaphragmatic hernia (CDH) that impact the prognosis of survival and long-term morbidity.
- In the prenatal setting, advances in genetic testing have made routine implementation of exome sequencing (ES) possible to identify monogenic disorders.

What does this study add?

- This is the first study performed in a clinical setting describing the added diagnostic yield of ES in prenatally isolated CDH or complex CDH (associated structural anomalies).
- In 10% of isolated CDH cases and 17% of complex CDH cases, we observed pathogenic or likely pathogenic single nucleotide variants.

- These findings support the broader use of ES in CDH, even in fetuses or neonates with CDH as an apparently isolated anomaly.

1 | INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a life-threatening condition that is generally diagnosed by fetal ultrasound in the second trimester.[1] Survival rates have improved over the years and are influenced by several factors, such as gestational age (GA) at birth, side of the defect (left-sided or right-sided), severity of pulmonary hypoplasia, associated structural anomalies (isolated or complex) and/or genetic abnormalities.[2,3]

More than 100 syndromes are reportedly associated with CDH, and given their impact on postnatal outcomes, genetic testing of the fetus is always advised.[1] In many of these syndromes, multiple structural anomalies are present. Some of these associated anomalies are subtle, such as dysmorphic features, or become apparent later, such as developmental delays. Genetic testing has evolved significantly in recent decades, with copy number variant (CNV) analysis with genomic microarrays largely replacing classical karyotyping for chromosomal analysis. In addition, recent advances in processing times and cost reductions have made the routine implementation of exome sequencing (ES) in the prenatal setting possible.[4] The added yield of diagnosing monogenic disorders provides expecting parents with the most detailed genetic information. The classification of variants (pathogenic, likely pathogenic, of unknown significance, likely benign or benign) is time-consuming, and there is a risk of incidental findings (IF) unrelated to the observed ultrasound abnormalities.[5] As such, unbiased and extensive pretest counseling to address the possible outcomes and limitations of genetic testing is a prerequisite and essential for parents to make an informed choice.[4] The Erasmus MC University Medical Center (Erasmus MC) in Rotterdam and the Radboud university medical center (Radboudumc) in Nijmegen are the two expertise centers for CDH in the Netherlands. Prenatal ES was recently introduced as part of routine care and offered to expecting parents in case of ultrasound abnormalities.

The aim of this study was to evaluate the diagnostic yield of ES in fetuses and neonates with isolated or complex CDH on prenatal examination and normal CNV analysis in a nationwide cohort.

2 | PATIENTS AND METHODS

2.1 | Study population

For this retrospective cohort study, we included all consecutive pregnancies with fetal CDH referred for prenatal ultrasound at the Department of Obstetrics and Gynecology of the Erasmus MC or the Radboudumc between January 2019 and June 2022. In these tertiary expertise centers for CDH, prenatal ultrasound was performed by experienced sonographers. CDH was diagnosed after a first-trimester anomaly scan (11–14 weeks gestational age [GA]), a 20-week anomaly scan or an ultrasound scan (US) in the third trimester.

2.2 | Diagnostic workflow

When CDH was suspected on prenatal ultrasound, the expectant parents were offered invasive testing through either chorionic villus sampling or amniocentesis. Chorionic villus sampling was performed between 11^{+0} and 14^{+0} weeks GA, and amniocentesis was offered for 15^{+0} weeks GA onward. If parents declined prenatal testing, postnatal genetic testing was offered, in which case umbilical cord blood or infant peripheral blood was used.

Rapid aneuploidy detection (RAD) was performed with Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) (Devyser Compact V3 (Devyser) for Erasmus MC or Aneufast Multiplex QF PCR kit v4 (Genomed Diagnostics AG) for Radboudumc). If the RAD was normal, the parents were seen by a clinical geneticist for pretest counseling on the available genetic tests (CNV and singe nucleotide variant [SNV] analysis). If parents opted for SNV in addition to CNV analysis, both analyses were performed simultaneously with either ES or with a microarray (CNV) and an ES (SNV).[6] To avoid findings in genes that are not yet associated with congenital anomalies, bioinformatic panel filtering for variants in a broad prenatal multigene panel, that is, genes associated with congenital abnormalities and/or intellectual disability, was performed in Erasmus MC. In Radboudumc, parents were offered the bioinformatic filter for the "Mendelian Inheritance in Men" (OMIM) gene panel with or without open exome analysis if negative. Nevertheless, while performing broad gene panel analysis, there is also a possibility of encountering pathogenic or likely pathogenic variants that may not explain the phenotype or match the indication for testing. Various terms (i.e., IF, unexpected findings, and unexpected diagnosis) have been used to describe these variants. Not only were variants explaining the fetal phenotype discussed with the parents, but IF were also discussed. In practice, national recommendations regarding the disclosure of IF in the postnatal setting are being followed. These guide towards disclosure of pathogenic variants associated with early onset and/or treatable disorders, while variants associated with an untreatable late-onset disorder are not disclosed.[7] The carrier status of a recessive disease allele is revealed only when a couple has at least a 25% risk of affected offspring. Parents' preferences other than this default are discussed during pretest counseling and noted on the consent form.

2.3 | Genetic analysis

2.3.1 | Erasmus MC

For CNV analysis, a SNP array (Illumina Infinium GSA + MD-24 v3 BeadChip, Illumina) was used, and analysis was performed with a resolution of 0.15 Mb. To facilitate interpretation of fetal CNVs, trio analysis of the fetus and both parents were performed for all cases. Trio ES was performed using Agilent SureSelect DNA + Human All Exon V7 capture and paired-end sequencing on the Illumina platform (outsourced). The average coverage of the exome was ~50x. The data were demultiplexed with bcl2fastq Conversion Software from Illumina. Reads were mapped to the genome (hg19) using the Burrows-Wheeler Aligner-Maximal Exact Match algorithm.[8] Variants were detected with the Genome Analysis Toolkit (GATK) Haplotype Caller.[9] The detected sequence variants were filtered and annotated with Alissa Interpret software and classified with Alamut Visual. Variants were further selected based on inheritance models (*de novo* autosomal dominant, autosomal recessive, X-linked recessive and filtering in a panel of imprinted genes).

2.3.2 | Radboudumc

ES and data analysis were performed as described previously,[6,10,11] with the use of the Human Core Exome Kit and extended RefSeq targets (Twist Biosciences) for enrichment and sequencing on a NovaSeq-6000 instrument (Illumina). Read alignment was performed using BWA, and variant calling was performed with GATK (SNVs), CoNIFER and ExomeDepth (CNVs). After that, variants were annotated using an in-house developed pipeline.[10] A simultaneous SNV and exome-wide CNV analysis from the ES data was executed with a bioinformatic filter for SNV variants in genes of our OMIM gene panel, after which variants were selected and prioritized (information about gene panel version and content and prioritization is available upon request). If no pathogenic or likely pathogenic variant was identified in the gene panel and only if parental consent was available, the bioinformatic filter was removed and analysis of the complete exome (open exome analysis) was performed. In the prenatal setting, a trio-analysis was preferably performed with both the fetus and parents to optimize variant prioritization; however, if parental DNA was not available or in the postnatal setting, a single analysis could be performed. If no exome-wide CNV analysis could be performed, an SNP array (CytoScan HD, Thermo Fisher) was executed with a resolution of 100 kb on fetal DNA and preferably both parents.

2.4 | Outcome

The primary outcome was genetic variants that were classified as pathogenic or likely pathogenic for the congenital anomalies according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (AMP) (ACMG/AMP) 2015 guidelines (SNV) and Silva et al. (2019) (CNV).[12,13] The following fetal and neonatal information was extracted from electronic patient records: GA at diagnosis, type of CDH (left-sided CDH (L-CDH) or right-sided CDH (R-CDH)), severity of pulmonary hypoplasia (determined by use of the observed to expected lung-to-head ratio (O/E LHR) and liver position),[2] associated anomalies, mode of delivery, pregnancy outcome (termination of pregnancy, intrauterine fetal death, live birth or neonatal death) and the course of neonatal care.

3 | RESULTS

During the study period, a total of 133 cases with CDH (n = 74 at Erasmus MC, n = 59 at Radboudumc) were referred for prenatal diagnosis and counseling. Among these 133 cases, 74% (98/133) had isolated CDH, and 26% (35/133) had complex CDH on prenatal examination (Figure 1). In the majority of cases (82%, 109/133), CDH was diagnosed after the second trimester anomaly scan. The minority was diagnosed in the first (4.5%, 6/133) or third trimester (13.5%, 18/133).

The majority of parents opted for genetic testing, that is 74% (73/98) in isolated cases and 94% (33/35) in complex cases. Amongst the isolated cases, invasive prenatal genetic testing was performed in 85% (62/73) and postnatal testing in 15% (11/73). This was, respectively, 82% (27/33) and 18% (6/33) in complex cases. In the isolated CDH group, three cases (4%, 3/73) had abnormal QF-PCR or CNV analysis results. Amongst complex CDH cases this percentage was 80% (12/15). The outcome was unknown for one isolated case due to loss to follow-up. These findings are summarized in Figure 1 and Supplemental Tables S1 and S2. After pre-test counseling, parents chose not to perform ES in 19 isolated cases and 3 complex cases with normal QF-PCR and CNV analysis. In total, ES analysis was performed in 50 isolated cases and 18 complex cases with normal QF-PCR and CNV results. In 10% (5/50) of the isolated cases and 17% (3/18) of the complex cases, a pathogenic or likely pathogenic SNV was found with ES analysis, resulting in a total diagnostic yield of 12% (8/68). Six of these pathogenic variants were causative for CDH (Table 1), and two pathogenic variants were causative for the associated anomalies but not (yet) known to be causative for CDH (Table 2).

3.1 | Isolated cases with pathogenic or likely pathogenic variants associated with CDH

The first case (#1) was referred at almost 21 weeks GA, and this fetus showed an isolated L-CDH with moderate pulmonary hypoplasia (O/E LHR 36.7%). Amniocentesis was performed, and ES analysis revealed a heterozygous pathogenic variant in the *RARB* gene (NM_001290216.2:c.1180C > T p.(Arg394Cys)), of which the mother was shown to be a low mosaic carrier (2/250 reads in plasma). This

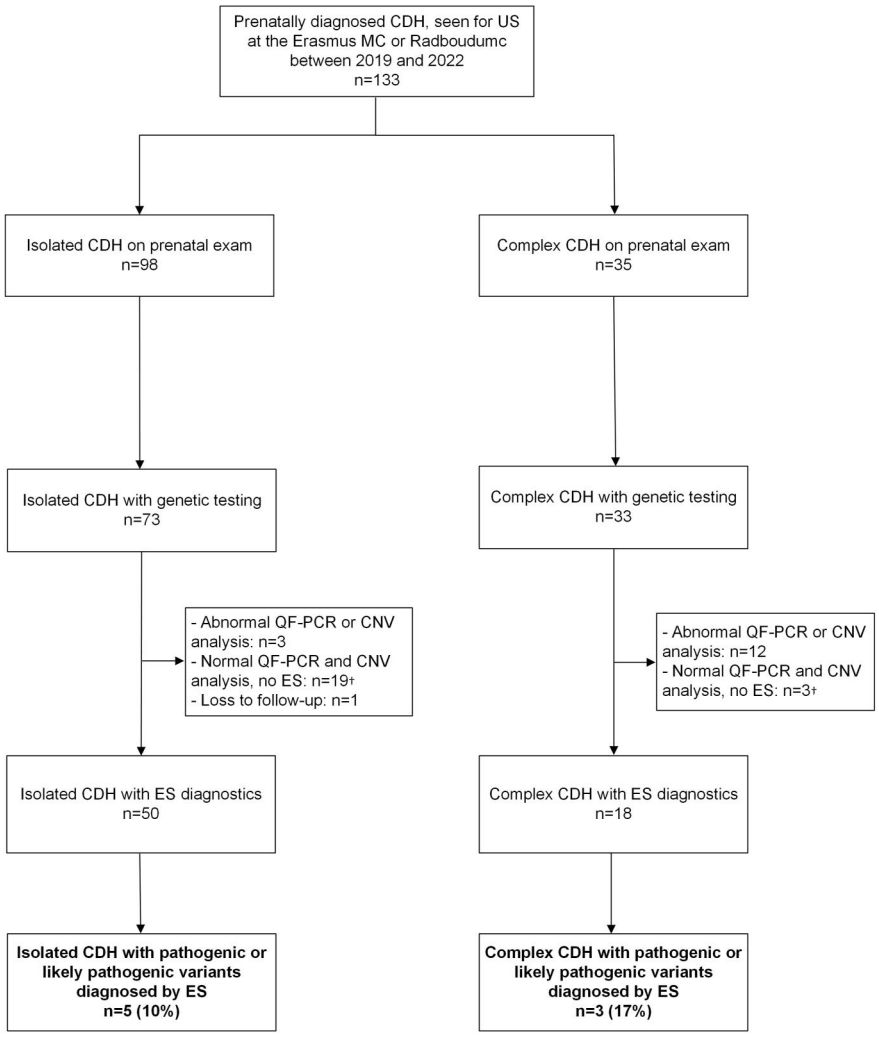

FIGURE 1 Flowchart of inclusions. CDH, congenital diaphragmatic hernia; ES, exome sequencing; US, ultrasound scan. † In 22 cases, parents refrained from ES analysis after pre-test counseling.

variant has previously been described in patients with microphthalmia syndrome with or without pulmonary hypoplasia and CDH (MIM #615524).[14] The parents decided to terminate the pregnancy, and the fetus was born at 23^{+6} weeks GA.

The second case was first evaluated in the second trimester (21 weeks GA) and also showed an isolated L-CDH with moderate pulmonary hypoplasia (O/E LHR 38.1%). ES diagnostics revealed a hemizygous maternally inherited variant of unknown significance in the *SMC1A* gene (NM_006306.3:c.598A > C p.(Lys200Gln)) in the male fetus, a gene associated with X-linked dominant Cornelia de Lange syndrome type 2 (MIM #300590), which was communicated with the parents. The pregnancy was continued, and an elective cesarean section was performed at 38 weeks GA. Postnatally, facial dysmorphisms characteristic of Cornelia de Lange syndrome were observed. After consultation with (international) experts, the clinical diagnosis of Cornelia de Lange syndrome was agreed upon. Considering the poor prognosis, palliative care was given, and the neonate died on day four of life. Methylation analysis of neonatal blood revealed a methylation pattern similar to that observed in Cornelia de Lange syndrome. Based on this latter information, the inherited variant was reclassified as a likely pathogenic variant.

The third isolated case (#3) was referred late in pregnancy, at approximately 35 weeks GA. Prenatally, this case was classified as an isolated R-CDH (O/E LHR 78%), but after birth, the neonate had a low birth weight in combination with microcephaly that were not detected on prenatal examination. Postnatal ES analysis revealed a *de novo* pathogenic variant in the *ZFPM2* gene (NM_012082.3: c.251C > G p.(Ser84*)) associated with CDH (MIM #610187). The child is alive and developing well.

3.2 | Complex cases with pathogenic or likely pathogenic variants associated with CDH

The first complex case (#4) was evaluated at approximately 15 weeks GA after an abnormal first-trimester anomaly scan. The fetus showed multiple congenital anomalies, including L-CDH (O/E LHR not measurable due to term and quality of imaging), a complex cardiac anomaly (i.e., a univentricular heart with truncus arteriosus) and a single umbilical artery. ES analysis revealed a *de novo* pathogenic variant in the *FGFR2* gene (NM_000141.4:c.755C > G p.(Ser252Trp)) associated with Apert syndrome (MIM #101200). The parents

TABLE 1 Pathogenic and likely pathogenic variants in genes associated with CDH identified with ES.

No	Isolated or complex CDH	Gene panel or open exome	Identified variant(s)	Applied ACMG/AMP criteria	Classification	Diagnosis	MIM
1	Isolated	Gene panel	*RARB* Chr3(GRCh37):g.25637919C > T NM_001290216.2:c.1180C > T p.(Arg394Cys); heterozygous, maternal mosaic	PVS1, PS2, PM2	Pathogenic	Microphthalmia syndrome	180,220
2	Isolated	Gene panel	*SMC1A* ChrX(GRCh37):g.53440199T > G NM_006306.3:c.598A > C p.(Lys200Gln); hemizygous, maternal	PS3, PM2,PP2, PP4_sup	Likely pathogenic	Cornelia de Lang syndrome 2	300,590
3	Isolated	Gene panel	*ZFPM2*; Chr8(GRCh37):g.106456559C > G NM_012082.3:c.251C > G p.(Ser84*); heterozygous, de novo	PVS1, PS2, PM2	Pathogenic	Diaphragmatic hernia 3	610,187
4	Complex	Gene panel	*FGFR2* Chr10(GRCh37):g.123279677G > C NM_000141.4:c.755C > G p.(Ser252Trp); heterozygous, de novo	PS2,PP2_sup, PM2_sup, PM5,PP3	Pathogenic	Apert syndrome	101,200
5	Complex	Gene panel	*SMARCA4* Chr19(GRCh37):g.11132512A > G NM_001128849.1:c.2728A > G p.(Thr910Ala); heterozygous, de novo	PM2, PP2, PP3, PS2	Pathogenic	Coffin-Siris syndrome	603,254
6	Complex	Gene panel	*USP9X* ChrX(GRCh37):g.41064648C > G NM_001039590.2:c.4917C > G p.(Tyr1639*); heterozygous, de novo	PVS1, PS2, PM2	Pathogenic	Intellectual developmental disorder, X-linked 99	300,919

Abbreviations: ACMG/AMP criteria, American college of medical genetics and genomics (ACMG) and the association for molecular pathology (AMP) criteria, MIM, Mendelian inheritance in Man; No, case number; PVS, pathogenic very strong; PS, pathogenic strong; PM, pathogenic moderate; PP, pathogenic supporting.

TABLE 2 Pathogenic and likely pathogenic variants in genes not (yet) associated with CDH identified with ES.

No	Isolated or complex CDH	Gene panel or open exome	Identified variant(s)	ACMG/AMP criteria	Classification	Diagnosis	MIM
7	Isolated	Gene panel	*ORC6L* Chr16(GRCh37):g.46723619T > C NM_014321.4:c.2T > C p.Met1?; heterozygous, paternal	PS1_strong, PVS1_sup, PM2_mod	Pathogenic	Meier-Gorlin syndrome 3	613,803
			ORC6L Chr16(GRCh37):g.46727099G > A NM_014321.4:c.449+5G > A p.? r.spl?; heterozygous, maternal	PM3_strong, PM2_mod, PP3_strong	Likely pathogenic		
8	Isolated	Gene panel	*PTPN11* Chr12(GRCh37): g.112888150A > G NM_002834.3: c.166A > G p.(Ile56Val); heterozygous, maternal	PS2_strong, PS4, PP2_sup, PP3_supPM2_mod, PM1_mod, PM5_mod,	Pathogenic	Noonan syndrome	163,950

Abbreviations: ACMG/AMP criteria, American college of medical genetics and genomics (ACMG) and the association for molecular pathology (AMP) criteria, MIM, Mendelian inheritance in Man; No, case number; PVS, pathogenic very strong; PS, pathogenic strong; PM, pathogenic moderate; PP, pathogenic supporting.

decided to terminate the pregnancy (birth at approximately 17 weeks GA).

The second case (#5) was referred at approximately 18 weeks GA. Several fetal anomalies, including L-CDH with moderate pulmonary hypoplasia (O/E LHR 29.2%), upper and lower limb anomalies (syndactyly, brachydactyly and clinodactyly), a duplex renal collecting system, a round skull, a flat facial profile with prominent lips and abnormal cavum septi pellucidi, were detected. ES analysis revealed a *de novo* pathogenic variant in the *SMARCA4* gene (NM_001128849.1: c.2728A > G p.(Thr910Ala)) associated with Coffin–Siris syndrome (MIM #614609). The parents decided to continue the pregnancy, and after a spontaneous preterm delivery, the neonate died on the first day of life after receiving palliative care.

Case #6 was referred at approximately 30 weeks GA and diagnosed with a complex L-CDH with mild pulmonary hypoplasia (O/E LHR 66.4%). Associated anomalies included ventriculomegaly and suspected midfacial hypoplasia. Late amniocentesis was performed at 30 + 2 weeks GA, and ES analysis revealed a *de novo* pathogenic variant in the *USP9X* gene (NM_001039590.2:c.4917C > G p.[Tyr1639*]), which leads to X-linked dominant, female-restricted,

intellectual developmental disorder type 99 (MIM # 300968). The parents opted for a late termination of the pregnancy at approximately 35 weeks GA. Recently, a paper showing CDH as a component of the anomalies associated with pathogenic variants in the *USP9X* gene was published.[15]

3.3 | Cases with pathogenic variants not (yet) associated with CDH

In two L-CDH fetuses, pathogenic variants were found with ES analysis that were known to be causative for other anomalies that may or may not have been visible with prenatal ultrasound but not (yet) known to be associated with CDH. The first case (#7) was diagnosed at the 20-week US with an isolated L-CDH and was found to have compound heterozygous pathogenic and likely pathogenic variants in the *ORC6* gene (NM_014321.4:c.2T > C p.Met1? and NM_014321.4:c.449+5G > A p.? r.spl? respectively) associated with Meier-Gorlin syndrome type 3 (MIM #613803). Meier–Gorlin syndrome type 3 is characterized by microtia, patellar aplasia/hypoplasia, and short stature, all of which are not (yet) visible on the anomaly scan. The parents opted for a termination of the pregnancy before 24 weeks GA.

The final case (#8) was referred at approximately 16 weeks GA and was diagnosed with isolated L-CDH (O/E LHR 40.1%). Amniocentesis was performed at the referring center. ES analysis revealed a pathogenic variant in the *PTPN11* gene (NM_002834.3:c.166A > G p.(Ile56Val)) associated with Noonan syndrome (MIM #163950). The variant was inherited from the mother, who turned out to have a mild phenotype. The neonate was born alive at approximately 38 weeks GA but died on day 13 of life due to persistent pulmonary hypertension not responding to maximal therapy.

4 | DISCUSSION

We present the diagnostic yield when adding rapid ES in the workflow of genetic testing for fetuses and neonates who were prenatally diagnosed with an apparently isolated or complex CDH. In 12% of cases with normal CNV analysis results that underwent extended genetic testing with ES, a pathogenic or likely pathogenic SNV was found. These variants were found in eight different genes; the majority (75%) were known to be causative (*SMC1A*, *RARB*, *ZFPM2*, *FGFR2*, *SMARCA4* and *USP9X*) for all the anomalies, including CDH, and a smaller number (25%) were causative for the associated abnormalities but not (yet) for CDH.

The reported diagnostic yield of adding SNV analysis with ES to CNV analysis is variable, and in general, it appears to be greater for multisystem anomalies.[16] For isolated anomalies, the observed percentage of genetic variants varies considerably, for example, from 4.5% to 11.5% in heart anomalies[17–19] to over 80% in skeletal dysplasias.[20–23] CDH has a strong association with genetic abnormalities, particularly when there are additional structural defects. The excellent performance of ES was previously described in a retrospective analysis of 76 complex CDH patients, in which pathogenic variants were identified in 37% of those patients.[24] These higher numbers can be explained by the fact that the study concerns a selected population of complex CDH patients in which there was a greater likelihood of underlying genetic pathology. Our study has been conducted in a clinical setting where the a priori chances of underlying genetic pathology, especially due to the number of apparently isolated CDH cases, are lower than in a selected population with complex CDH cases. We regard this as a strength of the study as it offers valuable guidance in the prenatal counseling of both apparently isolated and complex CDH.

Confirmation of a genetic cause for the observed ultrasound anomalies is particularly important in determining the recurrence risk. Additionally, by identifying a genetic cause, we can provide parents with a clearer prediction of the postnatal (long-term) outcomes of their unborn child. In cases where the impact is very severe or incompatible with independent life, neonatal palliative care could be considered in consultation with parents. It is also important when expensive (novel) treatments such as fetal therapy or extracorporeal membrane oxygenation are considered, as in those instances, the benefits of such an intervention should be weighed against the background of a genetic abnormality. However, whether it aids parents in their decision-making process about the continuation of pregnancy depends on their own opinions and beliefs. The presence of major associated structural abnormalities may already be a decisive factor for parents.[25] However, in isolated cases, this information may certainly have significant implications for prenatal and postnatal management. Herein, we observed relevant variants in an extra 10% of seemingly isolated CDH cases when adding ES to CNV analysis (abnormal CNV in isolated cases: n = 3/73 cases, 4%). Our data are consistent with what was previously reported when *post hoc* ES was performed in a cohort of 120 isolated cases (10% pathogenic variants).[26] This raises doubts about the practicality of selecting cases based on the fetal phenotype, as the presence of pleiotropy, the subtle nature of certain syndromic features or undetected fetal growth problems can sometimes render prenatal detection unfeasible.[27,28] This was highlighted by the cases diagnosed with *SMC1A*, *RARB* and *ZFPM2* gene variants in this series, which were all isolated during prenatal ultrasound.

The association of variants in *ORC6* and *PTPN11* with CDH may be a coincidence, although an association between *PTPN11* and CDH has been published before.[29] In contrast, several cases of CDH and pathogenic variants in the *USP9X* gene have been reported, and the mouse homolog of USP9X is expressed in the developing mouse diaphragm.[15]

We cannot entirely exclude certain selection bias, first because some parents decided not to undergo genetic testing. Second, although CDH care is centralized in two academic centers in the Netherlands, there may be some complex cases in which the parents decided to discontinue the pregnancy prior to referral. The validity of our findings will need to be confirmed in larger series requiring international collaboration. Such opportunities will soon become

available with further reductions in processing times and costs for both ES and genome sequencing.

5 | CONCLUSION

To our knowledge, this is the first study performed in a clinical setting describing the added diagnostic yield of routine use of rapid ES in cases of prenatally detected CDH with normal CNV analysis results. We observed pathogenic or likely pathogenic variants in a considerable portion of cases (12%), but more importantly, not only in complex CDH cases. Our findings support the broader use of ES in CDH cases, even in fetuses with CDH as an apparently isolated condition.

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None.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All variants of clinical interest are available in tables and supplementary tables.

ETHICS STATEMENT

Because subjects are not being submitted to any handling, nor are there rules of human behavior being imposed, Institutional Review Board approval was waived by the ethical committee of the Erasmus MC, Rotterdam, The Netherlands (MEC-2022-0641).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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