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# Prenatal diagnosis of fetal digestive system malformations and pregnancy outcomes at a tertiary referral center in Fujian, China: A retrospective study

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ABSTRACT

Fetal digestive system malformations (DSMs) are correlated with chromosomal anomalies. The prenatal diagnosis of DSMs allows for timely treatment and reduces perinatal morbidity and mortality. However, genetic screening for fetal DSMs is rarely reported. This study aimed to investigate genetic etiology and pregnancy outcomes in cases of fetal DSM by analyzing correlations between DSM types and chromosomal anomalies. This retrospective single-center study included 126 fetuses in whom DSMs were detected via prenatal ultrasonography. Genetic etiology was investigated using conventional karyotyping, chromosome microarray analysis (CMA), and whole-exome sequencing (WES). DSMs were categorized as simple DSM (Group A), DSM combined with abnormal ultrasound soft markers (Group B), and DSM combined with comorbidities of other systems (Group C). Abnormal karyotypes were detected in 11/126 (8.7 %) fetuses. Four more pathogenic copy number variants (CNVs) were detected using CMA, increasing the detection rate to 11.9 %. The detection rates significantly differed between the three DSM types (1.78 %, 8.11 %, and 33.33 % in Groups A, B, and C, respectively). The overall adverse pregnancy outcome rate was 33.9 %, and 11.5 %, 23.5 %, and 81.3 %, ($P < 0.001$), respectively, in Groups A, B, and C. Out of 83 live births, three neonates died, 26 underwent postnatal surgery with 24 favorable outcomes, and 54 did not undergo surgery and were basically normal. Two neonates who underwent WES were diagnosed with *CHD7*-associated Charge syndrome and *JAG1*-associated Alagille syndrome, respectively. Our findings demonstrate that fetal DSM is closely related to

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chromosome aneuploidies, CNVs, and point mutations. The prognoses of most fetuses with simple DSM and those with comorbid abnormal ultrasound soft markers were favorable in the absence of chromosomal anomalies and severe structural malformations, provided they underwent timely surgery as neonates. These findings provide guidance for the prenatal diagnosis and clinical management of fetal DSMs and the genetic counseling of parents.

## 1. Introduction

Fetal digestive system malformations (DSMs) account for approximately 7.35 % of all congenital deformities [1], and their diagnoses are largely based on prenatal ultrasound findings in the second and third trimesters. The main ultrasonographic manifestations of DSMs include digestive tract stenosis or atresia (esophageal, duodenal, and anal), intestinal duplication, meconium ileus or peritonitis, and hepatobiliary malformations. The etiological factors of abnormal fetal digestive systems include genetic factors, maternal smoking, exposure to infections, anemia, and environmental factors [2–4]. The prenatal diagnosis of DSMs allows for aggressive surgical treatment of neonates before serious complications occur after birth. This reduces perinatal morbidity and mortality to as low as 5 % in the case of duodenal atresia [5,6]. However, prognoses are poor if other body system anomalies or chromosomal abnormalities are also present.

Previous studies have revealed that gastrointestinal tract malformations are correlated with common chromosomal anomalies, among which duodenal stenosis/atresia and esophageal atresia have the closest correlation with trisomy 21 and trisomy 18, respectively [7,8]. With the wide use of chromosome microarray analysis (CMA) in genetic testing [9], copy number variants (CNVs), including microdeletion and microduplication, have been described in individuals with congenital digestive tract malformations, such as 17q22-q23.3 deletion in esophageal atresia [10], 17q11.2 deletion/duplication [11,12] and 4q22.3 deletion [7] in duodenal atresia, 13q32.1-q33.3 deletion in anal atresia [13], and 6p21.32 and 22q11.21 CNVs in Hirschsprung disease [14], among others. However, reports on genetic screening for fetal DSMs are rare. In this study, we aimed to investigate the prenatal diagnosis and clinical outcomes in 126 DSM cases detected via prenatal ultrasonography. Our findings provide references for the prenatal diagnosis, genetic counseling, and clinical management of women bearing fetuses with malformations of the digestive system.

**Table 1**
Demographic characteristics for 126 pregnancies with fetal digestive system malformations.

| Groups | Group A[a] (n = 56) | Group B[b] (n = 37) | Group C[c] (n = 33) | Total |
|---|---|---|---|---|
| **Maternal age (years)**, (Range, Mean ± SD) | 21–40, 30 ± 4 | 24–42, 31 ± 4 | 21–37, 29 ± 4 | 21–42, 30 ± 4 |
| **Gestation age at fetal digestive system anomalies initially diagnosed** (Range, Mean ± SD) | $18–34^{+5}$, 26 ± 4 | $18^{+2}–32^{+3}$, 26 ± 3 | $19^{+3}–32^{+6}$, 25 ± 4 | $18–34^{+5}$, 26 ± 4 |
| **Sample** | | | | |
| Amniotic fluid | 29 | 20 | 17 | 66 |
| Cord blood | 27 | 17 | 16 | 60 |
| **Type of digestive system anomalies** | | | | |
| Absent gallbladder | 12 | 8 | 6 | 26 |
| Abnormal gastric bubbles | 3 | 8 | 9 | 20 |
| Duodenal ileus | 10 | 3 | 5 | 18 |
| Jejunal or ileal atresia/stenosis | 4 | 3 | 1 | 8 |
| Meconium peritonitis | 2 | 5 | 1 | 8 |
| choledochal cyst | 6 | 1 | 1 | 8 |
| Intestinal duplication | 3 | 2 | 1 | 6 |
| Esophageal atresia/stenosis | 3 | 1 | 1 | 5 |
| Intrahepatic calcification | 2 | 2 | 1 | 5 |
| Abdominal masses | 2 | 2 | 1 | 5 |
| Colonic atresia and stenosis | 3 | 0 | 1 | 4 |
| Hepatomegaly/splenomegaly | 1 | 1 | 2 | 4 |
| Hepatic hemangioma | 1 | 1 | 1 | 3 |
| Intrahepatic portosystemic venous shunt | 1 | 0 | 1 | 2 |
| Anal atresia | 1 | 0 | 0 | 1 |
| Meconium ileus | 1 | 0 | 0 | 1 |
| Urorectal septum malformation sequence | 0 | 0 | 1 | 1 |
| Esophageal diverticulum | 1 | 0 | 0 | 1 |

[a] Group A was referred as fetuses with simple digestive system malformations.

[b] Group B was referred as fetuses with digestive system malformations combined with abnormal ultrasound soft markers, including increased nuchal translucency, nuchal cystic hygroma, increased nuchal fold, cerebral ventriculomegaly, mild renal pelvis separation, echogenic intracardiac focus, hyperechogenic bowel, fetal femur/humerus length, head circumference/biparietal diameter less than gestational age, abnormal nasal bone development, choroid plexus cyst, posterior fetal fossa pool enlargement, single umbilical artery, venous catheter blood flow spectrum abnormalities, aberrant subclavian artery, polyhydramnios, oligohydramnios, etc.

[c] Group C was referred as fetuses with digestive system malformations combined with comorbidities of other systems, including nervous system, face and neck, respiratory system, cardiovascular system, urinary system, skeletal system, etc.

## 2. Materials and methods

### 2.1. Participants

Fetal samples were collected from 126 pregnant women (mean age, 30 ± 4 years; range, 21–42 years) who provided written, informed consent to undergo prenatal diagnostic examinations for fetal DSMs at Fujian Provincial Maternity and Children's Hospital (Fujian, China) between June 2015 and December 2020. The fetuses were prenatally diagnosed with DSMs at 18–$34^{+5}$ weeks of gestation (mean gestational age, 25 ± 4 weeks) via ultrasound examinations. Eighteen disease entities were included. The top 10 disease entities were: absent gallbladder (n = 26), abnormal gastric bubbles (n = 20), duodenal ileus (n = 18), jejuno-ileal atresia (n = 8), meconium peritonitis (n = 8), choledochal cyst (n = 8), intestinal duplication (n = 6), esophageal obstruction (n = 5), intrahepatic calcification (n = 5), and abdominal masses (n = 5), with these disease entities accounting for 109 (86.5 %) of the 126 fetuses. The other disease entities comprised colonic atresia and stenosis (n = 4), hepatomegaly/splenomegaly (n = 4), hepatic hemangioma (n = 3), intrahepatic portosystemic venous shunts (n = 2), anal atresia (n = 1), meconium ileus (n = 1), urorectal septum malformation sequence (n = 1), and esophageal diverticulum (n = 1). DSMs were grouped based on ultrasound findings as follows: Group A (n = 56) was characterized by simple DSMs; Group B (n = 37) was characterized by DSMs combined with abnormal ultrasound soft markers, including increased nuchal translucency, nuchal cystic hygroma, increased nuchal fold, cerebral ventriculomegaly, mild renal pelvis separation, echogenic intracardiac focus, hyperechogenic bowel, fetal femur/humerus length, head circumference/biparietal diameter less than gestational age, abnormal nasal bone development, choroid plexus cyst, posterior fetal fossa pool enlargement, single umbilical artery, venous catheter blood flow spectrum abnormalities, aberrant subclavian artery, polyhydramnios, and oligohydramnios, among others; and Group C (n = 33) was characterized by DSMs accompanied by other malformations, including face and neck malformations and nervous, respiratory, cardiovascular, urinary, and skeletal system malformations (Table 1).

### 2.2. Sample collection

Informed consent was obtained from parents. Amniotic fluid was obtained via amniocentesis at 17–$23^{+6}$ gestational weeks, or umbilical cord blood was obtained via cordocentesis at over 24 weeks. The gestational week was determined based on the last menstrual period and ultrasonography findings obtained at 11–$13^{+6}$ weeks. Genomic DNA was extracted using a QIAamp DNA Blood Mini kit (QIAGEN, Germany).

### 2.3. Karyotyping

Karyotype analysis was performed for all cases. Cells from samples were cultured and prepared for G-banding. Karyotype analysis and descriptions were based on the International System for Human Cytogenetic Nomenclature (ISCN 2016).

### 2.4. Chromosome microarray analysis

CMA was performed in 112 of the 126 cases. CNVs and loss of homozygosity (LOH) were detected using a genome-wide high-resolution single nucleotide polymorphism (SNP) array on a 750 k platform (Affymetrix, Santa Clara, CA, USA) containing 200 000 SNP markers and 550 000 CNV markers. All procedures were performed according to the manufacturer's protocol. The raw data were analyzed using Chromosome Analysis Suite (ChAS) version 3.1 software (Affymetrix), and CNVs and LOHs were annotated based on the GRCh37/hg19 Genome Build (July 2013). CNVs were finally classified as (1) pathogenic, (2) likely pathogenic, (3) variants of uncertain significance (VOUS), (4) likely benign, or (5) benign, following the guidelines of the American College of Medical Genetics (ACMG) [15,16]. The laboratory reported CNVs ≥100 kb of pathogenic/likely pathogenic and ≥400 kb of VOUS. In cases that presented abnormal microarray findings, parental testing was performed, if possible, to determine the inheritance pattern of the deletion or duplication. All CNVs were analyzed at a resolution of 100 kb from 50 markers.

### 2.5. Whole-exome sequencing

To detect potential pathogenic variants, WES was performed after obtaining parental consent. DNA was extracted, randomly fragmented, purified, and enriched to construct DNA libraries. The DNA libraries were sequenced using a NextSeq500 System sequencer (Illumina, San Diego, CA, USA) following the manufacturer's protocol. The called variants were annotated with the reference sequence GRCh37. For the extraction of significant mutations from the called variants, the considered criteria included the following: clinical findings presented in the proband by ultrasound and/or computed tomography and the variant's possible effect on gene function, frequency in the general population, and inheritance pattern in the trio analysis (if parental samples were available). Collectively, gene mutations were interpreted according to the ACMG guidelines (2015) [17].

### 2.6. Database search

CNV and gene mutation results were interpreted using the following online databases: PubMed (https://pubmed.ncbi.nlm.nih.gov/), Online Mendelian Inheritance in Man (OMIM; http://www.omim.org/), DECIPHER (http://decipher.sanger.ac.uk), University of California Santa Cruz Genome Browser (http://genome.ucsc.edu/), ClinGen Dosage Sensitivity Curations (https://search.

clinicalgenome.org/kb/gene-dosage?page=1&size=25&search = ), 1000 Genomes Project (http://browser.1000genomes.org/), Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org/), Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php).

### 2.7. Pregnancy outcomes and postnatal follow-up

Follow-up was conducted from October 2020 to December 2020. The results of postnatal ultrasound examinations, pregnancy outcomes, and postnatal growth and development were followed up by telephone and electronic clinical information at our hospital.

### 2.8. Statistical analysis

SPSS software version 24.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. $P < 0.05$ was considered statistically

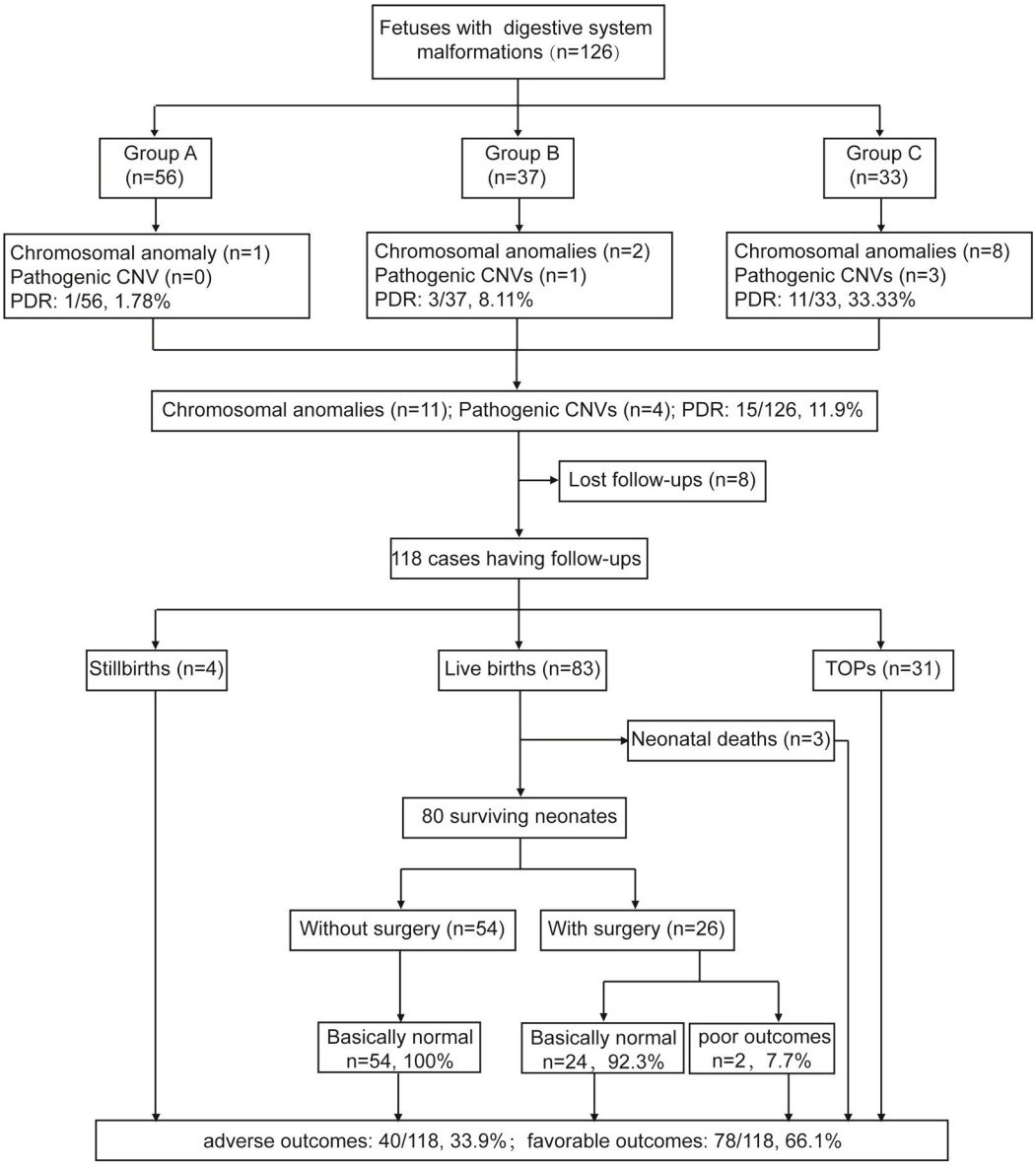


**Fig. 1. Overall flow of analysis in our study.** Group A, fetuses with simple digestive system malformations; Group B, fetuses with digestive system malformations combined with abnormal ultrasound soft markers; Group C, fetuses with digestive system malformations combined with comorbidities of other systems. CNV, copy number variant; PDR, positive diagnostic rate; TOP, termination of pregnancy.

significant.

## 3. Results

### 3.1. Prenatal diagnosis of fetal digestive system malformations

A flow chart of the overall analysis is depicted in Fig. 1. Conventional karyotyping was performed in all 126 cases. Abnormal karyotypes were detected in 11 (8.7 %) cases, including three cases of trisomy 21, seven cases of trisomy 18, and one case of der(21). The remaining 115 (91.3 %) cases exhibited normal karyotypes. Typical karyotyping results for cases 2, 96, 101, and 105 are shown in Fig. 2. In addition to the chromosomal anomalies detected by karyotyping in 11 cases, four pathogenic CNVs were identified including one 17q12 deletion syndrome (case 89), one 22q11.2 deletion syndrome (DiGeorge syndrome) (case 103), one Miller–Dieker syndrome (case 120), and one 22q11.2 duplication syndrome (case 126) (Fig. 3), increasing the detection rate to 11.9 % (15/126). In total, the detection rates in Groups A, B, and C were 1.78 % (1/56), 8.11 % (3/37), and 33.33 % (11/33), respectively, with a significant intergroup difference ($P < 0.001$). Table 2 presents the abnormal genetic results obtained in these 15 cases. Out of these cases, induction of labor was performed in 13 cases.

In case 2, der(21) was detected using karyotyping, and CMA revealed a concurrent 14.3-Mb duplication at 2p25.3p24.3, containing 50 protein-coding genes but no triplosensitive genes, and 573-kb deletion at 21q22.3. The 21q22.3 deletion contained *DIP2A* (607711) and another 10 OMIM genes. The haploinsufficiency score for *DIP2A* was 1 as shown in the ClinGen database. Termination was performed in this case due to the pathogenic CNVs and prenatal ultrasound findings (small bowel obstruction), despite the absence of genes or CNV syndromes associated with small bowel obstruction. Labor was induced in the cases involving DiGeorge syndrome (case 103) and Miller–Dieker syndrome (case 120). In the cases involving 17q12 deletion syndrome (case 89) and 22q11.2 duplication syndrome (case 126), development continued, and the pregnancies were full-term. During the follow-up of the 17q12 deletion

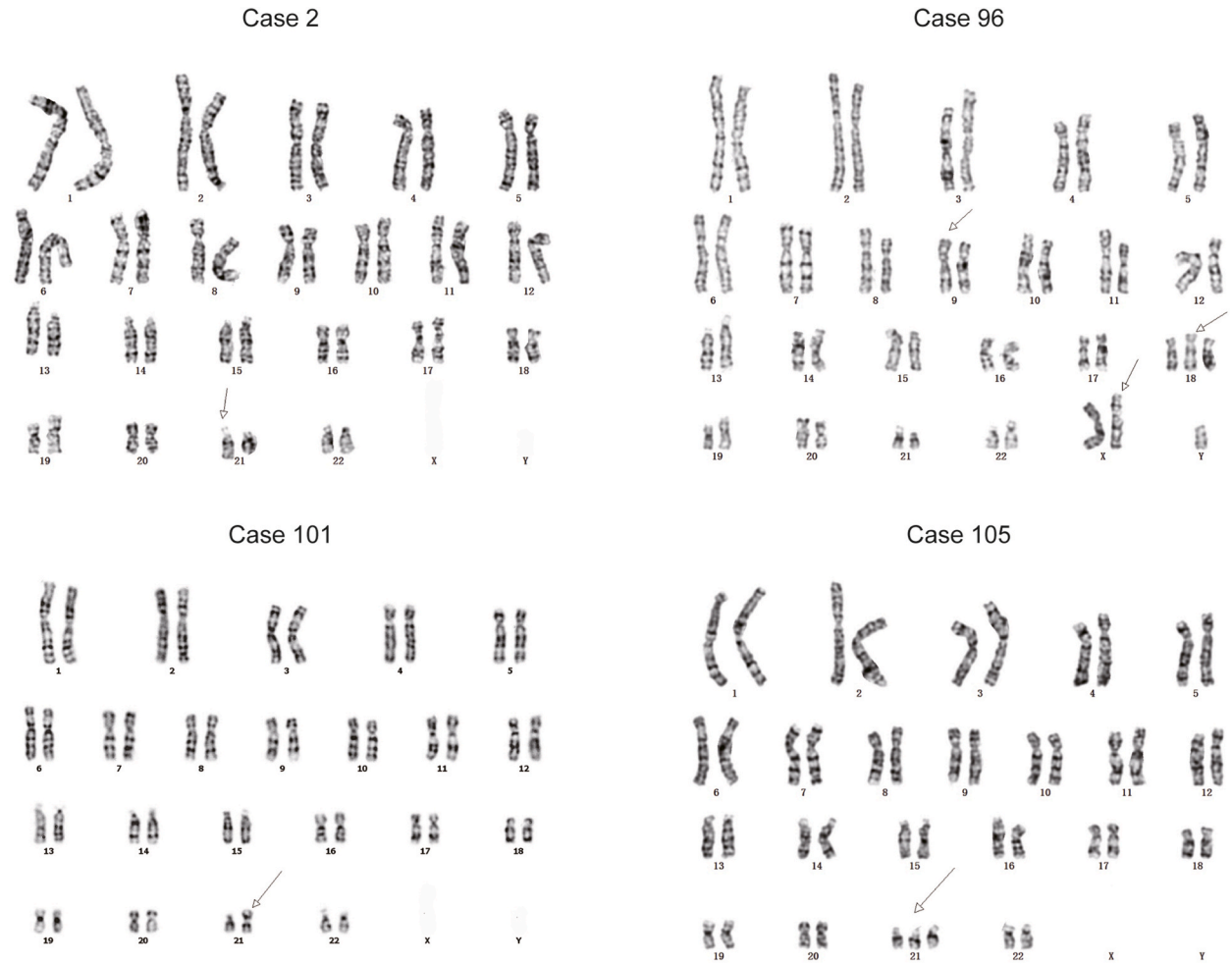


**Fig. 2. Typical karyotyping results of four cases.** Case 2, 46,XN,der(21)t(2;21)(p24.3;q22.3); Case 96, 48,XXY,inv(9)(p12q13),+18; Case 101, 46,XN,rob(21)(q10;q10); Case 105, 47,XN,+21. The arrow points to an abnormal chromosome.

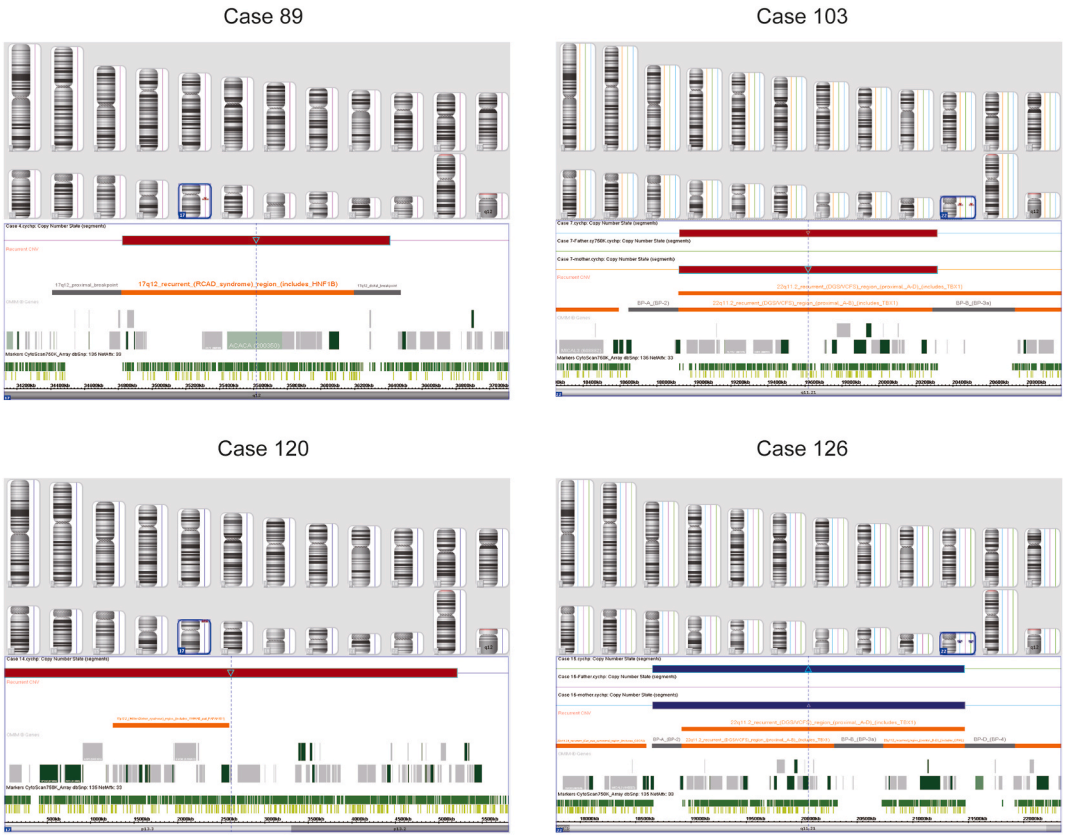


**Fig. 3. Four cases found with copy number variants (CNVs) by chromosomal microarray analysis.** Red and blue bars represent copy number deletions and duplications, respectively. Case 89, arr[GRCh37]17q12(34822465–36404555) × 1; Case 103, arr[GRCh37] 22q11.21 (18919477–20312661) × 1; Case 120, arr[GRCh37]17p13.3p13.2(5525–5204373) × 1; Case 126, arr[GRCh37]22q11.21(18,648,855–21459713) × 3. Parental testing was performed for case 89 and case 126, each with maternally inherited CNV.

syndrome (case 89) at 14 months of age and 22q11.2 duplication syndrome (case 126) at 18 months of age, respectively, normal development was exhibited for each.

VOUS were detected using CMA in three cases (Table S1). One fetus (case 83) carried a 2.2 Mb deletion that encompassed two OMIM genes, *LINGO2* (609793) and *MIR873* (616137), that had no related disorders. Ultrasonography in late pregnancy had indicated an abnormal frequency spectrum of blood flow signals in the middle cerebral artery, fetal growth restriction, and polyhydramnios. The neonate died five days after birth. Multiple LOHs were detected in another fetus (case 89); termination of pregnancy was performed due to malformations detected via ultrasound, i.e., bilateral ventricle broadening (1.4 mm) greater than gestational age and suspected small intestinal obstruction. These multiple LOHs occurred on different chromosomes, the percentage of which was 3.6 %, as estimated by the sum of the sizes of the homozygous segments (>5 Mb) divided by the total marker-covered autosomal length (~2781 Mb). The third fetus (case 113) carried a duplication at 16p13.11, and this pregnancy was also terminated due to the urorectal septum malformation sequence detected via ultrasonography.

In three cases (cases 46, 80 and 110), chromosomal balanced translocations or chromosomal inversions detected by karyotyping were undetectable by CMA (Table S2). In these three cases, termination was performed in case 110 due to the presence of multiple structural malformations (duodenal obstruction, smaller femur length, ventricular septal defect, and persistence of the left upper cavity) detected via prenatal ultrasonography.

We further investigated the correlation between different types of DSMs and chromosomal anomalies. As shown in Table 2, the prevalence of chromosomal anomalies was the highest (40.0 %; 8/20) in fetuses with abnormal gastric bubbles. Chromosomal anomalies were found in 16.7 % (3/18) and 12.5 % (1/8) of fetuses with duodenal ileus and small bowel obstruction, respectively. No chromosomal anomalies were observed in fetuses with esophageal or large bowel obstruction. One variant each was detected in eight fetuses with choledochal cyst, five intrahepatic calcifications, and two intrahepatic portosystemic venous shunts, respectively.

### *3.2. Pregnancy outcomes*

In total, 118 (93.5 %) of the 126 cases were followed up at ages ranging from 13 months to five years. Labor was induced in 26.3 % (31/118) of cases, intrauterine fetal death occurred in 3.4 % (4/118) of cases, and live births occurred in 70.3 % (83/118) of cases. Of

**Table 2**
Fifteen fetuses detected with genetic abnormalities by karyotyping or chromosome microarray analysis.

| Case No. | Group | Ultrasound presentation on digestive system | Other findings | Karyotyping | CMA (GRCh37) | Classification of variants | Pregnancy outcome | Follow-up |
|---|---|---|---|---|---|---|---|---|
| 2 | A | Small bowel obstruct-tion, meconium peritonitis | None | 46,XN,der(21)t (2;21)(p24.3; q22.3) | 2p25.3p24.3 (12770–14313,160) × 3; 21q22.3 (47,520,595–48093,361) × 1 | P, VOUS | TOP | NA |
| 58 | B | Duodenal obstruction | Nasal bone dysplasia, overlapping fingers | 47,XN,+21 | (21)x3 | P | TOP | NA |
| 76 | B | Unclear gastric bubble | Left lateral ventricle diameter was at high limit of normal value | 47,XN,+18 | (18)x3 | P | TOP | NA |
| 89 | B | Large gastric bubble | Increased echo of bilateral renal parenchyma, polyhydramnios | Normal | 17q12 (34822465–36404555) × 1 | P | Term birth | Normal development |
| 96 | C | Small gastric bubble | Cervical lymphatic cyst, overlapping fingers, single umbilical artery | 48,XXY,inv(9) (p12q13),+18 | (X)x2, (Y)x1, (18)x3 | P | TOP | NA |
| 101 | C | Intrahepatic multiple small nodules and enhanced parenchymal echo | Enhanced echo of bilateral renal corte, posterior fetal fossa pool enlargement, right heart larger than left heart, mild tricuspid regurgitation | 46,XN,rob(21) (q10;q10) | (21)x3 | P | TOP | NA |
| 103 | C | Choledochocele | Cardiac dysplasia, permanent right umbilical vein | Normal | 22q11.21 (18919477–20312661) × 1 mat | P | TOP | NA |
| 104 | C | Absent gastric bubble, large gallbladder | Tetralogy of Fallot, small jaw, bilateral ventricular choroid plexus cyst | 47,XN,+18 | (18)x3 | P | TOP | NA |
| 105 | C | Duodenal obstruction | Bilateral cerebral ventriculomegaly, partial corpus callosum hypoplasia, increased nuchal fold, nasal bone dysplasia, ventricular septal defect, right subclavian artery vagus | 47,XN,+21 | (21)x3 | P | TOP | NA |
| 106 | C | Absent gastric bubble | Absent bladder, suspected neural tube malformation, absent cerebellomedullary cistern, umbilical hernia, abnormal posture of hands, single umbilical artery, nasal bone loss, increased nuchal translucency | 47,XN,+18 | (18)x3 | P | TOP | NA |
| 107 | C | Absent gastric bubble | Ventricular septal defect, hook hand, left rocking chair foot | 47,XN,+18 | (18)x3 | P | TOP | NA |
| 108 | C | Duodenal atresia/stenosis | Ventricular septal defect | 47,XN,+18 | (18)x3 | P | TOP | NA |
| 119 | C | Small gastric bubble | Bilateral choroid plexus cyst, micromandible, short nasal bone, ventricular septal defect, widened inferior vena cava, bilateral radius loss, bipedal varus | 47,XN,+18 | (18)x3 | P | TOP | NA |
| 120 | C | Small gastric bubble | Bilateral cerebral ventriculomegaly, bipedal varus, cerebellar vermis dysplasia, polyhydramnios | Normal | 17p13.3p13.2 (5525–5204373) × 1 | P | TOP | NA |
| 126 | C | Intrahepatic portosystemic venous shunt | Tumor-like expansion of the ventral segment of umbilical vein, fetal growth restriction | Normal | 22q11.21 (18,648,855–21459713) × 3 mat | P | Term birth | Normal development |

CMA: chromosome microarray analysis; P, pathogenic, VOUS, variants of uncertain significance; TOP, terminal of pregnancy; mat, maternally inherited. NA, not applied. Groups A, B, and C were previously described as Table 1.

the 83 live births, deaths occurred in three cases (cases 35, 83, and 95) without genetic anomalies. Duodenal ileus and small intestine stenosis or atresia were prenatally detected in case 35, and the anus was absent in the postnatal examination. Thus, treatment was withdrawn and the neonate died three days after birth. In another case (case 83) with small gastric bubbles, brain atrophy, and peritoneal effusion, death occurred five days after birth. Case 95 involved high-density umbilical cord coiling, type III esophageal atresia, irregular heart rate, reflexes, muscle tone, and respiration, and death occurred 11 days after birth. Furthermore, the accuracy of the detection of fetal DSMs using prenatal ultrasonography in 83 preserved pregnancies was investigated. As shown in Table S3, postnatal conditions that were inconsistent with prenatal ultrasound findings were present in 23 cases. The prenatal detection rate of duodenal ileus, small bowel obstruction, meconium peritonitis, intestinal duplication, esophageal obstruction, and intrahepatic calcification was >75 % for each.

Of the surviving 80 neonates, 26 underwent postnatal surgery resulting in two poor and 24 favorable outcomes. The remaining 54 neonates who did not undergo surgery were basically normal. The two cases involving poor outcomes included case 9 in which death occurred five months after congenital megacolon surgery and case 97 presenting with cholestasis, butterfly vertebrae, and developmental delay (Fig. 1; Table 3). Of note, in one case (case 92) included in the 24 cases with favorable outcomes, two surgeries were performed: a bowel obstruction developed after surgery for congenital jejunal atresia type I, and a second surgical procedure was required. All neonates with a postnatal diagnosis of duodenal ileus, jejunoileal atresia/stenosis, or biliary atresia were surgically treated, and favorable outcomes were observed in the majority.

The overall rate of adverse pregnancy outcomes in the 118 followed-up patients was 33.9 %, and the adverse pregnancy outcome rate in Groups A, B, and C were 11.5 % (6/52), 23.5 % (8/34), and 81.3 % (26/32), respectively. The rate of labor induction was higher in Group C (65.6 %, 21/32) than in Groups A (7.7 %, 4/52) and B (17.6 %, 6/34) ($P < 0.001$). The difference in the rates of labor induction between Groups A and B was not significant ($P > 0.05$).

### 3.3. Whole-exome sequencing

WES was performed in two cases with prenatally normal CMA and karyotyping results (Table 4). The first case (case 95) constitutes one of the three neonatal deaths mentioned in section 3.2. A de novo pathogenic heterozygous mutation in *CHD7* (NM_017780: c.8956dupG) was detected. In the second case (case 97), a heterozygous mutation in *JAG1* (NM_000214:exon23:c.2895delT(p.F965Lfs*5) was detected. The mutation was classified as likely pathogenic considering that it is a frameshift mutation that results in loss of function. Cholestasis, butterfly vertebrae, and developmental delay were present at follow-up at the age of three years.

**Table 3**
The surgery option and clinical outcome for 83 live born infants.

| Clinical findings by prenatal ultrasound | No. of live born infants | Surgery treated | | No surgery treated | |
|---|---|---|---|---|---|
| | | Basically Normal | Poor outcome | Basically normal | Poor outcome |
| Absent gallbladder | 24 | 0 | 1 biliary atresia (Case 97)[a] | 23 | 0 |
| Abnormal gastric bubbles | 5 | 0 | 0 | 4 | 1 neonatal death (Case 83) |
| Duodenal ileus | 12 | 11 (Case 31)[b] | 0 | 0 | 1 neonatal death (Case 35)[c] |
| Jejunal or ileal atresia/stenosis | 4 | 4 | 0 | 0 | 0 |
| Meconium peritonitis | 8 | 3 | 0 | 5 | 0 |
| Choledochal cyst | 7 | 2 | 0 | 5 | 0 |
| Intestinal duplication | 2 | 1 | 0 | 1 | 0 |
| Esophageal atresia/stenosis | 4 | 1 | 0 | 2 | 1 neonatal death (Case 95) |
| Intrahepatic calcification | 4 | 0 | 0 | 4 | 0 |
| Abdominal masses | 2 | 0 | 0 | 2 | 0 |
| Colonic atresia and stenosis | 2 | 1 | 1 death (Case 9) | 0 | 0 |
| Hepatomegaly/splenomegaly | 2 | 0 | 0 | 2 | 0 |
| Hepatic hemangioma | 2 | 0 | 0 | 2 | 0 |
| Intrahepatic portosystemic venous shunt | 2 | 0 | 0 | 2 | 0 |
| Anal atresia | 1 | 1 | 0 | 0 | 0 |
| Meconium ileus | 1 | 0 | 0 | 1 | 0 |
| Urorectal septum malformation sequence | 0 | 0 | 0 | 0 | 0 |
| Esophageal diverticulum | 1 | 0 | 0 | 1 | 0 |

[a] Case 97 postnatally diagnosed with biliary atresia and then underwent surgery. The manifestations of cholestasis, butterfly, and developmental delay were presented at the age of 3 years.

[b] Case 31, one out of 11 cases prenatally diagnosed with duodenal ileus, was postnatally diagnosed with small bowel obstruction and then underwent surgery.

[c] Case 35, one out of 11 cases prenatally diagnosed with duodenal ileus, was postnatally diagnosed with small bowel obstruction and anal atresia, then this infant died 3 days after birth without surgery.

**Table 4**
Clinical data for two cases undergoing whole exome sequencing.

| Case No. | Clinical examinations | Gene | Variant | Associated phenotype | Inheritance pattern | Origin | Classification | Clinical outcome |
|---|---|---|---|---|---|---|---|---|
| 95 | Prenatal ultrasound showed esophageal atresia and pulmonary valve stenosis. Postnatal examinations confirmed esophageal atresia, and also found cardiac defect, external ear malformation, cryptorchidism and small penis | *CHD7* | Chr8: 61778453 NM_017780: c.8956dupG het | Charge syndrome | AD | De novo | Pathogenic | No surgery treated, and death occurred 11 days after birth |
| 97 | Prenatal ultrasound showed absent gallbladder and suspected thoracic hemivertebrae. Postnatal examination showed biliary atresia and small than gestational age (SGA) | *JAG1* | chr20:10622128 NM_000214: exon23: c.2895delT(p.F965Lfs*5) het | Alagille syndrome | AD | Not applied | Likely pathogenic | The child was diagnosed as Alagille syndrome, whose manifestations included cholestasis, butterfly vertebrae, ocular abnormalities and developmental delay |

AD, autosomal dominant inheritance; het, heterozygous.

## 4. Discussion

Previous studies have revealed that DSMs are closely associated with chromosome aneuploidies [7,8]. In this study, abnormal karyotypes were detected in 8.7 % of DSM cases, including cases of trisomy 21, trisomy 18, and der(21). These findings are similar to that of Martin et al. [18] who have reported abnormal karyotypes (primarily trisomy 21) in 8.88 % of pediatric patients with congenital DSMs exhibiting comorbid malformations of other systems. In addition, chromosomal anomalies were most prevalent in fetuses with abnormal gastric bubbles, which are often detected in combination with other abnormalities using prenatal ultrasonography. These results suggest that the incidence of chromosomal anomalies significantly increases when other system abnormalities accompany DSMs and highlight the importance of prenatal genetic testing.

In one case, we detected a mutation in *CHD7*, which causes Charge syndrome (MIM:214800) [19] via autosomal dominant inheritance. The esophageal atresia, heart defects, and external ear malformations manifested in this case fell within the phenotypic spectrum of this syndrome. In another case, a heterozygous mutation in *JAG1* was detected. *JAG1* is associated with an autosomal dominantly inherited disorder, Alagille syndrome (MIM: 118450) [20]. However, the variant identified in this case has not been previously reported in the literature or databases. The phenotypic manifestation in this case included biliary atresia and butterfly vertebrae, which are relatively consistent with the characteristics of Alagille syndrome. Surgery was performed to treat the biliary atresia.

We detected 17q12 deletion syndrome in one case and 22q11.2 duplication syndrome in another case. As previously described [21], individuals with 17q12 deletion syndrome, also known as renal cysts and diabetes syndrome, may present with abnormalities of the kidneys, maturity-onset diabetes of the young type 5, developmental delay or intellectual disability, duodenal atresia, and other variable features, with a clinical penetrance of 34.4 %. 22q11.2 duplication syndrome has a clinical penetrance of 21.9 % and is associated with clinical findings that may include global developmental delay, psychomotor retardation, learning difficulties, cardiovascular abnormalities, hypotonia, and dysmorphic features but DSMs have not been reported. As 17q12 deletion syndrome and 22q11.2 duplication syndrome show incomplete penetrance and variable expressivity, future follow-up will be needed in these cases. Overall, induction of labor was required in most cases exhibiting genetic abnormalities, and term pregnancies were reported after full genetic counseling in the remaining two cases with incomplete penetrance, suggesting the guiding value of prenatal genetic testing for prognosis evaluation and pregnancy outcomes.

Previous studies have reported individuals with partial trisomy 2p syndrome, with manifestations mainly including mental retardation, typical facial appearance, ocular anomalies, and scoliosis or kyphosis [22–24]. The 21q22.3 deletion may be associated with dysphasia (dyslexia). One study reported that in one pedigree, a father and son had deletions in the 21q22.3 region, including *PCNT*, *DIP2A*, *S100B*, and *PRMT2*, and the clinical phenotype was learning dyslexia [25]. In our study, the detection of both deletions and duplications on different chromosomes indicated that one parent carried a balanced translocation. The parents had normal karyotypes, thus further detection of cryptic balanced translocation by fluorescence in situ hybridization is recommended. In addition, we found chromosomal balanced translocations or chromosomal inversions detected by karyotyping that were not detected by CMA. In individuals with chromosomal balanced translocation or inversion, phenotypes are often normal; however, exceptions arise, such as when a functional gene is interrupted or with the formation of a fusion gene.

The VOUS detected in our study are most likely unrelated to DSMs and are considered incidental findings in genetic testing. In one case, the detection of multiple LOHs on different chromosomes indicated fourth-degree relatedness of the parents. However, the parents denied consanguinity and declined the opportunity to perform WES for the detection of underlying pathogenic variants. A duplication was detected at 16p13.11, which is a neurodevelopmental disorder susceptibility locus associated with developmental delay, cognitive impairment, and autism [26], with low clinical penetrance (approximately 7–8%).

Our results affirm the effectiveness of surgery to correct malformations of the gastrointestinal tract [5,6,27,28]. The postoperative prognoses of neonates with DSMs were generally good; only 1 neonate out of 26 neonates that were surgically treated died five months after colon surgery. The most common type of malformation in this study was congenital duodenal ileus, the prognosis of which was favorable in the absence of chromosomal abnormalities and other severe malformations outside the digestive system, provided that timely postpartum surgery was performed. Of note, the rate of chromosomal aneuploidies and induced labor was higher in Group C than in Groups A and B, demonstrating that chromosomal abnormalities often cause multiple malformations. Recently, next-generation sequencing (NGS) methods such as WES or whole-genome sequencing have been used in the diagnosis of genetic diseases, which can improve the molecular diagnostic yield for fetuses with structural anomalies and provide better clinical guidance [29,30]. Lord et al. [30] have reported a missense variant in *MYCN* that is associated with Feingold syndrome in a fetus with duodenal atresia detected via prenatal ultrasonography. However, no diagnostic variant has been reported in fetuses with gastrointestinal tract anomalies [29]. In our study, clinically relevant variants were detected in both neonates who underwent WES. Prenatal diagnoses could have offered the parents more comprehensive clinical prognostic evaluations.

We found inconsistencies between prenatal ultrasound findings and postnatal conditions, which may be associated with the level of skill of the ultrasound physician, the performance of Doppler ultrasound instruments, or the dynamic development of the fetus. Nonetheless, our results indicate a high detection rate of DSMs, suggesting that prenatal ultrasonography provides an important reference for parents to make an informed choice regarding either termination or continuation of the pregnancy and the selection of postnatal surgical treatment plans.

The limitations of our study should be noted. First, the data were obtained from a retrospective single-center study, and incomplete follow-up information might have led to the underestimation of late-onset adverse phenotypes. To avoid the loss of important postpartum information, especially in cases involving abnormalities, we advise parents to return to our hospital for appropriate examinations if abnormal symptoms are observed in children. Second, for the detection of an underlying single mutation, most patients did not undergo WES, which is mainly attributed to the limited use of NGS in prenatal diagnosis during the past five years. The use of NGS to prospectively detect variants associated with fetal DSMs is a priority for future studies.

## 5. Conclusions

Genetic testing of fetuses with DSMs via a combination of conventional karyotyping and CMA gave a diagnostic yield of 11.9 %, and postnatal WES revealed genetic mutations, suggesting the importance of genetic tests in the diagnosis of DSMs. The incidence of chromosomal anomalies significantly increased for DSMs accompanied by abnormalities of other systems. Advances in minimally invasive laparoscopic surgery allow pediatric patients with congenital DSMs without genetic disorders involving chromosomal abnormalities to undergo timely postnatal surgery that may improve prognoses.

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## Ethics approval

All procedures followed were in accordance with the ethical standards of the responsible institutional committee on human experimentation and with Helsinki Declaration of 1975 (revised in 2000). This study was approved by the Ethics Committee of the Fujian Maternity and Child Health Hospital (ID: No. 2021KLRD643). Written informed consents were obtained from all the participants.

## Data availability statement

Data will be made available on request.

## CRediT authorship contribution statement

**Bin Liang:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Data curation, Conceptualization. **Fang Yang:** Writing – original draft, Data curation, Conceptualization. **Hailong Huang:** Methodology, Investigation, Data curation. **Zhaozhen Liu:** Resources, Methodology, Data curation. **Qingqiang Ji:** Methodology, Data curation. **Yan Wang:** Methodology, Data curation. **Xiaoqing Wu:** Methodology, Data curation. **Yuan Lin:** Supervision, Investigation. **Lanting Xie:** Methodology. **Wantong Zhao:** Data curation. **Hua Cao:** Funding acquisition. **Liangpu Xu:** Writing – review & editing, Supervision, Data curation. **Na Lin:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## List of abbreviations

| | |
|---|---|
| DSMs | digestive system malformations |
| CMA | chromosome microarray analysis |
| CNVs | copy number variants |
| WES | whole-exome sequencing |
| LOH | loss of homozygosity |
| SNP | single nucleotide polymorphism |
| VOUS | variant of uncertain significance |
| TOP | termination of pregnancy |

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21546.

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