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RESEARCH ARTICLE

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Prenatal genetic detection in foetus with gallbladder size anomalies: cohort study and systematic review of the literature

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ABSTRACT

Objectives: The aim of the study was to evaluate the detection rate of genetic abnormalities in cases of foetal gallbladder (FGB) size abnormalities to determine whether these abnormalities justify prenatal diagnosis.

Methods: Two hundred and twenty-seven foetuses with gallbladder (GB) size anomalies who underwent prenatal diagnosis between January 2015 and June 2024 were included in the study. All these patients underwent chromosomal microarray and/or karyotyping, and 37 cases also underwent whole exome sequencing (WES). Two hundred and eight cases were followed up for postnatal outcomes. Then, we reviewed the literature of FGB anomalies cases with confirmed chromosomal results.

Results: The study included 227 foetuses, comprising 60 cases with isolated GB size anomalies and 167 cases with non-isolated GB size anomalies. Non-isolated GB size anomalies were associated with findings such as hyperechogenic bowel, ventriculomegaly, foetal growth restriction (FGR), cardiac anomalies, renal dysplasia and single umbilical artery. The overall diagnostic yield of genetic tests was 10.57% (24/227). Aneuploidies were identified in seven foetuses. Pathogenic/likely pathogenic copy number variations (CNVs) were found in nine foetuses, and α0-thalassemia in five foetuses. Additionally, three pathogenic single-nucleotide variants (SNVs) were detected through WES. Foetuses with non-isolated GB size anomalies showed a higher rate of detecting genetic abnormalities compared to those with isolated GB size anomalies, with a significant difference in statistical analysis (13.2% vs. 3.3%, $p = .033$, Chi-square test). A total of eight studies, involving 407 cases met the criteria for inclusion in the systematic review. Overall, 28 foetuses were identified to have chromosomal abnormalities (6.9%, 28/407).

Conclusions: This study indicates that parents of foetuses with GB size anomalies should be informed about the potential for aneuploidy, pathogenic CNVs and SNVs, and genetic testing should be recommended in cases of non-isolated foetal GB size anomalies.

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Gallbladder size anomalies; FGB; CNVs; SNVs

Introduction

The gallbladder (GB) develops in the fourth week of embryonic growth from the upper bud of the hepatic diverticulum on the ventral side of the primitive mid-gut and starts forming a cystic structure in the twelfth week [1]. As a result, the foetal GB can be easily seen during the second and third trimesters. Studies have shown that the ultrasonic detection rate of foetal GB at 14–16 weeks and 24–32 weeks of gestation was 99% and 95%, respectively [2,3]. He et al. identified a linear correlation between the diameter of the foetal gallbladder (FGB) and gestational age by measuring 670 normal FGBs [4]. Therefore, in some instances, the GB was not visible initially or its diameter is small, it can still develop normally as gestational age increases.

Foetal gallbladder size anomalies are uncommon in clinical settings, primarily consisting of non-visualization of the foetal gallbladder (NVFGB), enlarged FGB and small FGB. The most prevalent is NVFGB, characterized by the failure to detect the foetal GB in two or more consecutive ultrasound

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examinations within one week of pregnancy. NVFGB has an incidence of 0.1–0.15% [5] and is associated with GB dysplasia, biliary atresia, cystic fibrosis, chromosome anomalies and intestinal obstruction [5]. It is reported that the incidences of transient non-visualization, GB agenesis, biliary atresia, cystic fibrosis and chromosomal abnormalities of isolated NVFGB in western populations were 69.4%, 24.7%, 3.5%, 2.4% and 1.4%, respectively [6]. In contrast, these incidences in cases of non-isolated NVFGB with additional sonographic abnormalities were 18.2%, 23.1% and 20.4%, respectively [6,7]. Enlarged FGB is defined as a GB length exceeding the 90th percentile, while small FGB is defined as a length below the 10th percentile [4]. Enlarged FGB and small FGB are infrequent in clinical practice, and there is limited research on their correlation with chromosome abnormalities.

Previous studies have primarily focused on the predictive ability of prenatal ultrasonography in identifying FGB anomalies, leaving a gap in knowledge regarding foetuses undergoing prenatal genetic testing. There is ongoing debate about the association between foetal GB abnormalities and the need for genetic tests. Shen et al. suggested that the correlation between isolated NVFGB and aneuploidy was likely coincidental [8]. Di Pasquo et al. found that the rate of chromosomal abnormalities in isolated and non-isolated NVFGB was 1.9% and 20.4%, respectively [7]. Qian et al. reported an overall detection rate of 5.9% for CMA-related BTS anomalies in foetuses [9]. Therefore, the aim of this study was to evaluate the detection rate of genetic abnormalities in cases of FGB size abnormalities to determine whether these abnormalities justify prenatal diagnosis.

Materials and methods

Cohort study

Cases with FGB size anomalies detected by ultrasound whose parents chose to undergo invasive prenatal diagnosis between January 2015 and June 2024 were collected in this study. The study was approved by our Institutional Review Board and Clinical Research Ethics Committee and it conforms to the Declaration of Helsinki. Written informed consent was obtained from the pregnant women.

Definitions used in the study were as follows: (1) NVFGB refers to the inability to observe the FGB in two or more consecutive ultrasound examinations within one week during pregnancy. (2) Enlarged FGB was defined as a GB length greater than the 90th percentile, while small FGB was defined as a length less than the 10th percentile. (3) Cases initially diagnosed as NVFGB but found to have a small GB in the third trimester/magnetic resonance imaging (MRI) were classified as having small GBs. The inclusion criteria of the cases in this study were that the foetus had any of the above GB abnormalities and the pregnant woman had undergone invasive prenatal diagnosis, and the exclusion criteria were that the foetus had the above abnormalities but had not undergone invasive prenatal diagnosis.

After the prenatal detection of FGB size anomalies in the second trimester, a systematic sonographic assessment for associated anomalies was conducted. Fetuses were considered isolated if no other associated anomalies, including soft markers or structural abnormalities, were observed. When combined with other ultrasound anomalies, it was categorized as non-isolated FGB size anomalies.

Genetic testing

Quantitative fluorescence polymerase chain reaction (QF-PCR) was commonly utilized to identify maternal cell contamination and promptly detect abnormal numbers of chromosomes 13/18/21 and sex chromosomes. Karyotype analysis was conducted to identify aneuploidies.

Detection of common α- and β-thalassemia was conducted in cases accompanied by hydrops fetalis, with the identification of the common six types of α-thalassemia mutations and 17 types of β-thalassemia mutations in southern China using a suspension array system as previously reported [10]. This kit was developed by our laboratory based on the barcode magnetic bead technology (http://www.apbiocode.com/) of Applied BioCode (Santa Fe Springs, CA). The specific experimental steps and instruments needed can be referred to the literature [10].

Genomic DNA was extracted using a DNA extraction kit (QIAamp DNA Mini Kit, QIAGEN, Hilden, Germany). CMA was performed using a whole-genome CytoScan 750K array (Thermo Fisher Scientific, Waltham, MA), as recommended by the manufacturer. According to standardized procedures, 250 ng DNA was digested, ligated, amplified, purified, fragmented, labelled and probe hybridized, which was washed in phosphate buffer and detected using a laser scanner. The raw data were analysed with the Chromosome Analysis Suite 4.0 (Thermo Fisher Scientific, Waltham, MA) based on the genome version GRCh37/hg19. The data were analysed in accordance with the guidelines of the American College of Medical Genetics.

CNV-seq was performed as described in our previous study with minor modifications [11] in 19 cases.

Briefly, 100–350 ng of genomic DNA was fragmented and DNA libraries were constructed by adapter ligation and PCR amplification. The DNA library was sequenced on an Ion Proton Sequencer (Thermo Fisher Scientific, Waltham, MA), yielding approximately 4–5 million raw single-ended sequenced reads of approximately 200 bp length. Using the Burrows–Wheeler algorithm, 2.5–3.5 million uniquely mapped reads were aligned with the genome version GRCh37/hg19 and assigned to a 20 kb bin on each chromosome. Three steps of loess regression, temporal normalization and linear model regression were used to eliminate the influence of GC bias among different samples. The cyclic binary segmentation (CBS) algorithm was used to identify CNVs.

Trio-whole exome sequencing (WES) was carried out in 37 cases with non-isolated GB anomalies. Genomic DNA was extracted as previously described. SureSelectXT Clinical Research Exome kit (Agilent Technologies, Santa Clara, CA) was used to prepare the library and enrich the targets as following the manufacturer's instructions [12]. Trio WES was performed using 2 × 150 bp in the paired-end mode of the NextSeq 500 platform (Illumina, San Diego, CA) with an average coverage of over 110×, with 97.6% of target bases covered at least 10×. Genetic results analysis was based on the genomic variation database (http://dgv.tcag.ca/dgv/app/home), DECIPHER database (https://decipher.sanger.ac.uk/) and OMIM database (http://www.ncbi.nlm.nih.gov/omim). The identified variants were further confirmed by Sanger sequencing.

Follow-up

All pregnancies underwent comprehensive follow-up through telephone interviews or review of patients' medical records post-amniocentesis to inquire about pregnancy outcomes. The main contents of follow-up were whether the foetus was born, mode of delivery, gestational age at birth, birth weight, and whether the foetus was normal.

Systematic review

To better assess the need for prenatal diagnosis in foetuses with GB size anomalies, we conducted a literature review of previous cases of prenatal genetic testing. The meta-analysis was performed according to protocol recommended for systematic reviews [13]. Then, we conducted a search on PubMed for cases of foetal GB anomalies with confirmed chromosomal results, using combinations of the following keywords: 'fetal gallbladder anomalies', 'NVFGB', 'Enlarged GB', 'Small GB' and 'prenatal diagnosis'. Only full-text articles meeting the criteria were deemed eligible for inclusion, with a focus on articles published in English. We reviewed all abstracts and relevant data regarding study characteristics and chromosomal results, the reference lists of all included articles were reviewed for any additional reports. Only cohort studies were incorporated. Quality assessment of the included studies was performed using the Newcastle-Ottawa Scale for cohort studies [14].

Statistical analysis

Statistical analysis was conducted using SPSS version 27.0 for Windows (SPSS Inc., Chicago, IL). Quantitative variables are presented as mean ± standard deviation (SD) (e.g. maternal age and gestational age), while categorical variables are expressed as frequency and percentage (such as prenatal sampling, isolated and non-isolated FGB, genetic abnormalities and normal results, among others). The Chi-square test was utilized to evaluate differences in genetic results concerning various parameters compared to the background risk; a p value of <.05 was considered statistically significant.

Results

Study characteristics

Totally, 227 cases opted for invasive prenatal diagnosis, with 60 cases having isolated FGB size anomalies (28 cases of enlarged FGB, nine cases of small FGB and 23 NVFGB) and 167 cases having non-isolated FGB size anomalies (52 cases of enlarged FGB, 42 cases of small FGB and 73 cases of NVFGB) (Figure 1, Supplemental Data 1). One hundred and twenty-four foetuses received an ultrasonic diagnosis in the second trimester, and 103 received an ultrasonic diagnosis in the third trimester. The mean maternal age was 29.6 ± 5.3 years. The mean gestational age at diagnosis was 27.8 ± 3.4 weeks. Overall, 61.6% of prenatal sampling was performed following amniocentesis, and 38.3% from cordocentesis sampling. QF-PCR was performed in 179 cases, karyotype analysis was conducted in 129 cases, CMA/CNV-seq was conducted in 213 cases, common α- and β-thalassemia detection was performed in seven cases, and Trio WES was performed in 37 cases. Among all the cases, 11 cases were testing using a single method, 99 cases using two methods, 109 cases using three methods and seven cases using four methods (Supplemental Data 1).

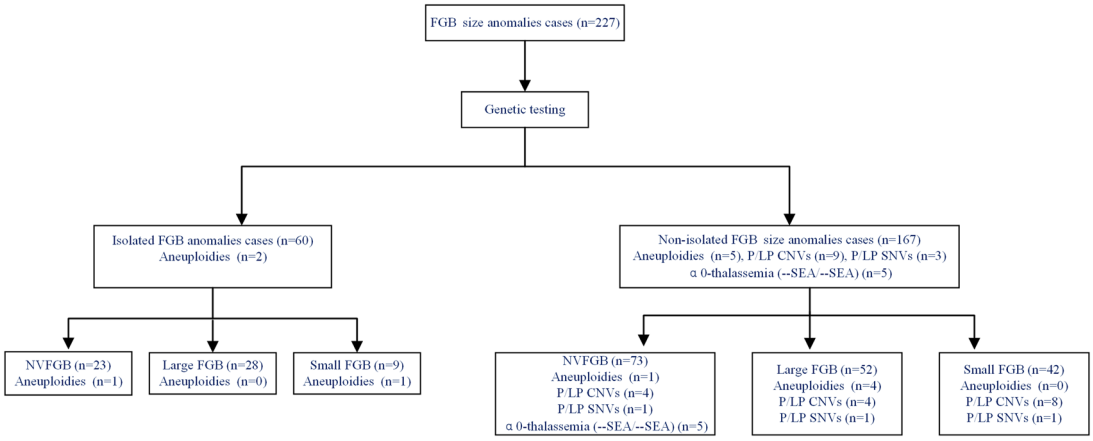

Figure 1. Prenatal diagnosis for fetuses with prenatally diagnosed with GB size anomalies.

Diagnostic yield of genetic testing

The genetic testing yield for foetuses with GB size anomalies was 10.57% (24/227). Aneuploidies were found in seven foetuses (3.08%, 7/227), including three trisomy 18 (T18) (1.3%, 3/227) (Supplemental Table 1), one Klinefelter's syndrome (0.4%, 1/227), one Turner syndrome (0.4%, 1/227), one 47, XXX (0.4%, 1/227) and one mos.47, XN, +9(7)/46, XN (0.4%, 1/227). Pathogenic/likely pathogenic copy number variations (CNVs) were detected in nine foetuses (3.96%, 9/227) (Supplemental Table 2), variants of unknown significance (VUS) were detected in 10 foetuses (4.4%, 10/227) (Supplemental Table 3). Additionally, α0-thalassemia (–SEA/–SEA) was identified in five foetuses (2.2%, 5/227) (Supplemental Table 4). Three pathogenic single-nucleotide variants (SNVs) were identified by WES (1.3%, 3/227) (Supplemental Table 5). In all, 18 genetic abnormalities were detected in the second trimester and six in the third trimester.

Nine pathogenic CNVs ranging from 4.5 kb to 6.46 Mb were identified, including 1p22.2p22.1 microdeletion, 1p36.33p36.31 microdeletion, 2q13 microdeletion, 16p12.2p11.2 microdeletion, 16p13.3 microdeletion, 17p12 microdeletion, 17q12 microdeletion and microduplication. Three pathogenic SNVs (pSNVs) in non-isolated FGB size anomalies were detected through WES, involving *KMT2D* (NM_003482) c.11851C>T, (p. Gln3951Ter); *PTEN* (NM_000314.8) c.635-1G>C, and *COL1A2* (NM_000089) c.3034G>A, (p. Gly1012Ser) (Figure 2).

Among 167 foetuses with non-isolated FGB size anomalies, there were five cases of aneuploidies, nine cases of pathogenic CNVs, five cases of α0-thalassemia (–SEA/–SEA) and three cases of pSNVs. The rate of genetic abnormalities was 13.2% (22/167). Isolated FGB size anomalies had two cases of aneuploidies, resulting in a genetic abnormality rate of 3.3% (2/60). Non-isolated FGB size anomalies showed a higher detection rate of genetic abnormalities compared to isolated cases, with a significant difference in statistical analysis (13.2% vs. 3.3%, $p = .033$, Chi-square test).

Outcome

We monitored the pregnancy outcomes of 227 foetuses with GB size abnormalities. Nineteen patients were not available for follow-up. Of the remaining 208 pregnancies, 25 mothers chose to terminate their pregnancies. There were 14 cases with genetic abnormalities, including three instances of T18, one instance of 45, X, five instances of alpha-0-thalassemia (–SEA/–SEA), three instances of pathogenic CNVs, two instances of pSNVs and 11 instances of non-isolated GB size abnormalities combined with other significant organ malformations and negative genetic tests. All the foetuses with isolated GB size anomalies chose to continue their pregnancies (Supplemental Data 1).

Systematic review

After conducting a search, a total of eight studies, excluding our cohort, involving 230 cases of NVFGB, 144 cases of large FGB and 33 cases of small FGB met the criteria for inclusion in the systematic review (Table 1). In total, 28 foetuses were identified to have chromosomal abnormalities (6.9%, 28/407). Out of 150 isolated NVFGB cases, three foetuses were found to have chromosomal abnormalities (2%, 3/150), and out of 77 non-isolated NVFGB cases, 14 were found to have chromosomal abnormalities (18.2%, 14/77). No chromosomal abnormality was found in 41 isolated large FGB cases (0%, 0/44), and in six out of 103 non-isolated large FGB cases (5.8%, 6/103). Additionally, no chromosomal abnormalities were found in seven isolated small FGB cases (0/7), and in five out of 26 non-isolated small FGB cases (5/26).

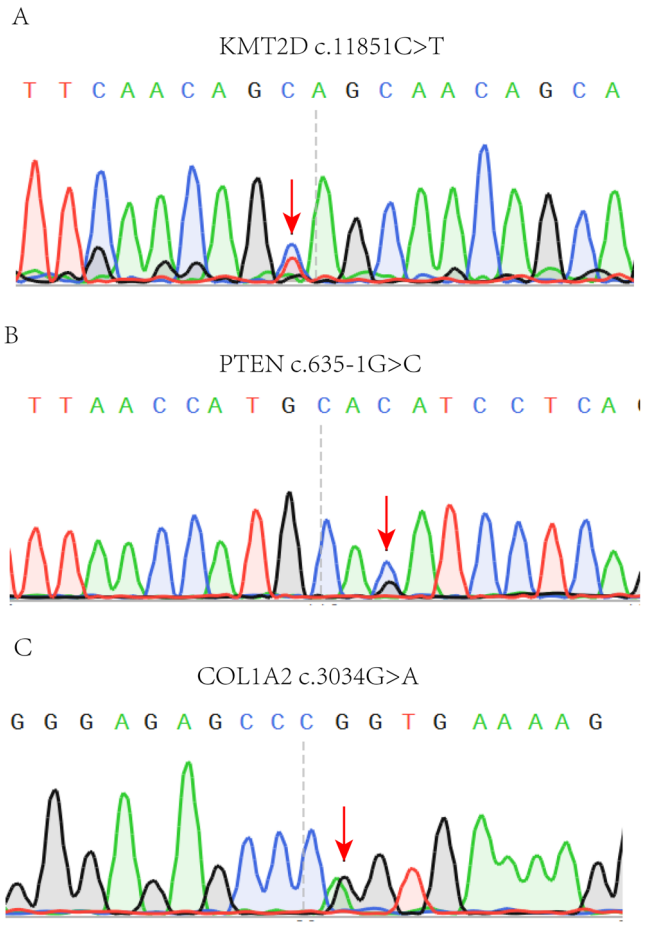

Figure 2. Sanger sequencing results for fetuses with pSNVs: (A) Sanger sequencing result of case 98 identified a pSNV in *KMT2D* (c.11851C>T; p. Gln3951Ter). (B) Sanger sequencing result of case 173 identified a pSNV in *PTEN* (c.635-1G>C). (C) Sanger sequencing result of case 204 identified a pSNV in *COL1A2* (c.3034G>A; p. Gly1012Ser).

Discussion

In our study, 227 samples underwent multiple genetic methods for prenatal diagnosis in foetuses with GB size anomalies. Additionally, we thoroughly evaluated pregnancy outcomes. The overall detection rate of genetic testing for FGB size anomalies in foetuses was 10.57% (24/227), including aneuploidy (3.08%, 7/227), P/LP CNVs (3.96%, 9/227), α0-thalassemia (–SEA/–SEA) (2.20%, 5/227) and P/LP SNVs (1.32%, 3/227). The detection rate of genetic anomalies in the total non-isolated group was significantly higher than that of the total isolated groups in foetuses with GB size anomalies. Follow-up results indicated better pregnancy outcomes in isolated groups compared to non-isolated groups.

Previous studies have sparked significant debate regarding the association between fetal GB abnormalities and the need for genetic testing. Although some researchers have detected chromosomal abnormalities in cases of FGB anomalies and suggested an association between FGB anomalies and chromosomal abnormalities [7–9], it is still unclear which chromosomal abnormalities are significantly correlated. In our study, chromosomal abnormalities were found in 16 foetuses (7.04%, 16/227), which aligns closely with the findings of our systematic review (6.9%, 28/407), further supporting the potential association between FGB anomalies and chromosomal abnormalities.

Our analysis of eight previous studies identified 230 pregnancies with NVFGB and confirmed karyotype results [5–9,15–17], comprising 153 pregnancies with isolated NVFGB and 77 pregnancies with non-isolated NVFGB. Among these, 17 cases (7.39%, 17/230) showed chromosomal abnormalities, including T18 (6); trisomy 21 (T21) (2); trisomy 13 (T13) (1); 7q11.23 microdeletion; 47, XX, +9[44]/46, XX [34]; 9p microduplication; 46, XX add(14)(q32.1); 16p11.2 microduplication; 22q11.2 microduplication; 47, XXX; 69, XXY. Three cases with chromosomal abnormalities (47, XXX, 16p11.2 microduplication and 14q32.1 duplication) were found in isolated NVFGB cases. These findings underscore T18 as the most prevalent chromosomal abnormality in the

Table 1. Main characteristics of studies reporting on fetal GB anomalies with confirmed chromosomal results, included in systematic review.

	Fetuses with NVFGB (*n*)		Fetuses with large FGB (*n*)		Fetuses with small FGB (*n*)		
Study	Isolated (*n*)	Non-isolated (*n*)	Isolated (*n*)	Non-isolated (*n*)	Isolated (*n*)	Non-isolated (*n*)	Main findings
Ochshorn et al. [15]	17/1	0	–	–	–	–	17 cases of NVFGB were evaluated in this study, and one chromosomal abnormality was found.
Shen et al. [8]	16/0	5/3	–	–	–	–	21 cases of NVFGB (16 isolated and five non-isolated cases) were included, three chromosomal abnormalities were found in non-isolated cases, and no abnormal karyotype was found in isolated cases.
Bardin et al. [16]	27/0	5/1	–	–	–	–	Among 32 cases of NVFGB (27 isolated cases and five non-isolated cases) in this study, a chromosomal abnormality was detected in the isolated case.
Sagi-Dain et al. [17]	45/1	6/2	–	–	–	–	Of 45 pregnancies with NVFGB, one pathogenic CNV was found. Of the six cases with non-isolated NVFGB, two chromosomal abnormalities were found.
Di Pasquo et al. [7]	10/0	6/0	–	–	–	–	Sixteen cases of NVFGB were followed in this study (10 isolated cases), and no chromosomal anomaly was reported.
Qian et al. [9]	30/0	44/5	41/0	103/6	7/0	26/5	271 pregnant women carrying fetuses with BTS abnormalities were included in this study, and 16 cases (5.9%, 16/271) of chromosomal anomalies were detected.
Ting et al. [6]	2/0	8/3	–	–	–	–	Among 19 cases of NVFGB in this study, amniocentesis/chorionic villi sampling were performed in 10 cases (eight non-isolated and two isolated cases); three chromosomal abnormalities were found in non-isolated cases, and no chromosomal abnormality was found in isolated cases.
Zhang et al. [5]	6/0	3/1	–	–	–	–	In this study, nine NVFGB patients agreed to undergo amniocentesis (six isolated and three non-isolated cases), one chromosomal abnormality was found in non-isolated case.

The numbers before the slash indicate the total number, and those after representing the cases of chromosomal abnormalities.

NVFGB population, highlighting the necessity for further exploration into the connection between T18 and NVFGB. In our research, we identified six cases (6.25%, 6/96) with chromosomal abnormalities among NVFGB cases. Of these, one case with 47, XXY was observed in isolated NVFGB cases, while chromosomal abnormalities were found in five non-isolated NVFGB cases. These included mos.47, XN, +9(7)/46, XN, 1p22.2p22.1 microdeletion, 1p36.33p36.31 microdeletion, 16p13.3 microdeletion and 17p12 microdeletion. These CNVs did not overlap with the CNVs identified in the literature review. Among these CNVs in our study, one case (DECIPHER patient: 395683) included in the Decipher database partially overlapped with the 1p36.33p36.31 microdeletion interval, and multiple abnormalities, such as GB abnormalities, were noted. Gallbladder abnormalities were not reported in DGV data for other CNVs. Therefore, the relationship between these CNVs and NVFGB warrants further investigation.

Studies focusing on large FGB are limited, with only one study mentioning the relationship between FGB enlargement and chromosome abnormalities. In the study by Qian et al., 144 cases of large FGB were collected, including 41 isolated cases and 103 non-isolated cases [9]. Among the non-isolated GB enlargement patients, six chromosomal abnormalities were detected (9p24.3p22.1 microdeletion, 17q12 microdeletion, 17p12 microdeletion, 2q36.1q37.3 microduplication, 7q11.23 microduplication and 22q11.21 microduplication), while no chromosomal abnormalities were found in the isolated group. This suggests that foetuses with isolated GB enlargement may not have an increased risk of total chromosomal anomalies. In our study, no chromosomal anomalies were observed in the isolated

FGB enlargement group, whereas seven foetuses in the non-isolated FGB enlargement group had chromosomal anomalies, including T18 (3), 45, X (1), 16p12.2p11.2 microdeletion (1), 17q12 microdeletion (1) and 2q13 microdeletion (1). No GB abnormalities were reported in Decipher database of theses CNVs. Therefore, these results further support the idea that isolated FGB enlargement may not be associated with chromosomal abnormalities.

Few studies have focused on the correlation between small GB and fetal chromosomal abnormalities. Qian et al. gathered 33 cases of small GB, including seven isolated small GB cases and 26 non-isolated small GB cases [9]. No chromosomal abnormalities were detected in the isolated small GB cases, while five chromosomal abnormalities (47, XXX, 1q21.1q21.2 microdeletion, 14q11.2q12 microduplication, 7q11.23 microdeletion and 20q13.2q13.33 microduplication) were identified in the non-isolated group. In our research, one pathogenic CNV (17q12 microduplication) (1/42) was observed in the non-isolated small GB group, and one case (1/9) with aneuploidy (47, XXX) was found in the isolated small GB group. Due to the limited number of studies and cases, the link between chromosomal abnormalities and fetal small GB remains unknown, necessitating more cases for further investigation.

The association between SNVs and FGB size anomalies is limited, with most studies focusing on GB atresia and cystic fibrosis. In our study, only one case presented with GB atresia and a normal karyotype, but no related gene testing was conducted. Additionally, five cases of severe thalassemia were identified in our patients, with no previous literature linking these abnormalities. Moreover, the incidence of thalassemia is high in our region, suggesting a potential accidental relationship. Three pSNVs in the genes *KMT2D*, *PTEN* and *COL1A2* were discovered in cases of non-isolated GB size anomalies through WES. pSNV in *KMT2D* was found in a case with NVFGB, aberrant right subclavian artery and renal dysplasia. *KMT2D* is associated with Kabuki syndrome, which is characterized by typical facial features (long palpebral fissures with eversion of the lateral third of the lower eyelid; arched and broad eyebrows; short columella with depressed nasal tip; large, prominent or cupped ears), minor skeletal anomalies, persistence of fetal fingertip pads, mild-to-moderate intellectual disability, and postnatal growth deficiency. Other findings may include congenital heart defects, genitourinary anomalies, cleft lip and/or palate, gastrointestinal anomalies including anal atresia, ptosis and strabismus, and widely spaced teeth and hypodontia [18]. In this study, the foetus's phenotype is part associated with Kabuki syndrome. pSNV in *PTEN* was found in a case with large FGB, ventriculomegaly, macrocephaly and polyhydramnios. *PTEN* is associated with Cowden syndrome (CS), Bannayan–Riley–Ruvalcaba syndrome and autism. The variant was reported in CS patients, which can result in the use of cryptic splice sites [19]. In this study, the foetus's phenotype is part associated with macrocephaly/autism syndrome. pSNV in *COL1A2* was found in a case with small FGB and skeletal dysplasia, and the variant is reported in patients with osteogenesis imperfecta (OI). It is well known that *COL1A2* is associated with OI [20], and the foetus phenotype is consistent with OI. Above all, the association between these genes and GB development has not been documented in OMIM database, PubMed and any other database, and these variations are primarily linked to additional structural abnormalities. Therefore, the connection between these gene variations and GB abnormalities is also likely coincidental.

We should consider the actual situation to provide appropriate recommendations when facing similar cases in clinical practice. For cases with isolated FGB size anomalies, chromosome analysis can be suggested; the prognosis of most isolated FGB size anomalies with a normal karyotype is good. For non-isolated FGB size anomalies, the detection method should be determined based on additional structural abnormalities and regional characteristics of the patient. When FGB size anomalies are combined with hydrops and thalassemia in an area with a high incidence, chromosome detection and thalassemia gene detection are recommended. When combined with other ultrasonic structural abnormalities, WES or other specific detection methods can be chosen.

The main limitation of the present study is its retrospective design, as the clinical data are from a single centre. Follow-up data long-term after childbirth were not available.

Conclusions

In summary, the study results show that genetic tests can enhance the detection of genetic abnormalities in foetuses with GB size anomalies. Counsellors should inform parents about the potential presence of pathogenic CNVs and SNVs in foetuses with GB size anomalies. Genetic testing is advisable for suspected antenatal GB size anomalies, particularly non-isolated cases.

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Author contributions

Yimo Zeng: investigation, methodology, analysis, writing—original draft, review and editing, visualization. Rong Hu, Jian Lu, Yiming Qi, Dan Chen and Chaoxiang Yang: Methodology and analysis; Jing Wu: conceptualization, supervision, review and editing. All authors have read and approved the final work.

Ethical approval

This study was approved by the Institutional Review Board/Medical Ethics Committee of Guangdong Women and Children Hospital (IRB reference number: 202301185).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

All data included in this study are available upon request by contact with the corresponding author.

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