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Optimal prenatal genetic diagnostic approach for posterior fossa malformation: karyotyping, copy number variant testing, or whole-exome sequencing?

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Abstract

Background Posterior fossa malformation (PFM) is a relatively uncommon prenatal brain malformation. Genetic diagnostic approaches, including chromosome karyotyping, copy number variant (CNV) testing, and whole-exome sequencing (WES), have been applied in several cases of fetal structural malformations. However, the clinical value of appropriate genetic diagnostic approaches for different types of PFMs has not been confirmed. Therefore, in this study, we aimed to analyze the value of different combined genetic diagnostic approaches for various types of fetal PFMs.

Methods This retrospective study was conducted at Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing Maternal and Child Health Care Hospital. Fifty-one pregnant women diagnosed with fetal PFMs who underwent genetic testing in our hospital from January 1, 2017 to December 31, 2022 were enrolled; women with an isolated enlarged cisterna magna were excluded. All participants were categorized into two groups according to the presence of other abnormalities: isolated and non-isolated PFMs groups. Different combined approaches, including karyotype analysis, CNV testing, and trio-based WES, were used for genetic analysis. The detection rates of karyotype analysis, CNV testing, and WES were measured in the isolated and non-isolated groups.

Results In isolated PFMs, pathogenic/likely pathogenic (P/LP) CNVs were detected in four cases (36.36%, 4/11), whereas G-banding karyotyping and WES showed negative results. In non-isolated PFMs, a sequential genetic approach showed a detection rate of 47.5% (19/40); karyotyping revealed aneuploidies in five cases (16.67%, 5/30), CNV testing showed P/LP CNVs in five cases (16.13%, 5/31), and WES identified P/LP variants (in genes *CEP20*, *TMEM67*, *OFD1*, *PTPN11*, *ARID1A*, and *SMARCA4*) in nine cases (40.91%, 9/22). WES showed a detection rate of 83.33% (5/6) in fetuses with Joubert syndrome. Only six patients (five with Blake's pouch cyst and one with unilateral cerebellar hemisphere dysplasia) survived.

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Conclusions We recommend CNV testing for fetuses with isolated PFMs. A sequential genetic approach (karyotyping, CNV testing, and WES) may be beneficial in fetuses with non-isolated PFMs. Particularly, we recommend WES as the first-line genetic diagnostic tool for Joubert syndrome.

Keywords Posterior fossa malformation, Karyotyping, Copy number variant, Whole-exome sequencing

Introduction

Posterior fossa malformation (PFM) is a spectrum of diseases that includes Dandy–Walker malformation (DWM), Blake's pouch cyst (BPC), vermian hypoplasia (VH), cerebellar hypoplasia (CH), mega cisterna magna, arachnoid cyst, rhombencephalosynapsis (RES), Joubert syndrome (JS), and other abnormalities [1]. The estimated incidence of PFMs during the neonatal period is ~ 1 in 5000 live births [2]. The relatively rare incidence of PFM and the wide range of PFM types lead to significant differences in prognosis [3].

Prenatal ultrasonography and magnetic resonance imaging (MRI) are screening and diagnostic tools for PFMs [3, 4]. Owing to the difficulty of definitively diagnosing the different PFM phenotypes, genetic testing has emerged as a powerful method for prognostic assessment and auxiliary prenatal diagnosis. Recently, various prenatal genetic diagnostic methods have been developed [5]. In addition, several genetic analyses have been conducted to identify chromosomal and genetic disorders. Traditional karyotype analysis, the most widely used method, can detect chromosome aneuploidy and structural rearrangement of large fragments; however, the approach is ineffective for detecting copy number variations (CNVs) < 5 Mb. Chromosomal microarray analysis (CMA) and low-pass whole genome sequencing (WGS) have become mainstream methods for prenatal genetic detection of CNVs, thus playing an essential role in chromosomal submicroscopic-level deletions and duplications. Although the combination of karyotyping and CNV testing can detect a series of numerical abnormalities, structural aberrations, and CNVs in chromosomes, a significant number of genetic causes of PFMs cannot be identified by this combined approach. However, the clinical application of whole-exome sequencing (WES) can detect the underlying genetic cause of 25–35% of birth defects with negative karyotype and CNV results; moreover, this approach provides additional genetic information to assist clinical counseling and obstetric management. However, the high cost of WES makes it a difficult option for doctors and parents [6].

At present, different research centers apply several genetic testing strategies for PFMs, with symptoms ranging from asymptomatic to severe neurological symptoms. Considering cost–benefit and prognostic factors, we recruited a relatively large sample of fetuses with PFMs to investigate the detection rate of multiple genetic diagnostic tools, including karyotyping, CNV testing, and WES, and establish an optimal strategy for the genetic diagnosis of PFMs.

Methods

Study setting and data sources

This retrospective analysis was conducted at Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing Maternal and Child Health Care Hospital. Pregnant women with PFMs who registered in our institution or were referred to our hospital between January 2017 and December 2022 were enrolled in this study. None of the probands had a family history of PFM or other abnormalities. The inclusion criteria were: (1) pregnant women with complete prenatal medical records (prenatal ultrasound and MRI) and (2) the pregnant woman and partner received detailed prenatal genetic counseling and provided peripheral blood samples. The exclusion criteria were: (1) incomplete prenatal clinical data and loss to follow-up and (2) pregnant women with fetal isolated mega cisterna magna (not associated with additional antenatal or postnatal MRI anomalies) [7, 8]. In total, 51 pregnant women were enrolled in this study. The mean age of the pregnant women was 31.67 ± 5.09 years (23–48 years). The mean gestational age at diagnosis was 23.57 ± 4.08 weeks (18^{+5}–36^{+5} weeks). The study profile is shown in Fig. 1.

Ethics statement

This study was approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital, Capital Medical University (Approval No. 2017-KY-076-01). Written informed consent was obtained from the study participants.

Sampling methods and phenotypic determination

In the present study, one patient underwent chorionic villus sampling, 29 underwent amniocentesis, and 21 underwent fetal blood sampling [9].

Fetuses of all enrolled participants underwent prenatal ultrasound assessment, and standard sections were conducted according to the International Society of Ultrasound in Obstetrics and Gynecology guidelines. Assessment of fetal PFM relies on the following planes: axial (transventricular, transcerebellar, and transthalamic

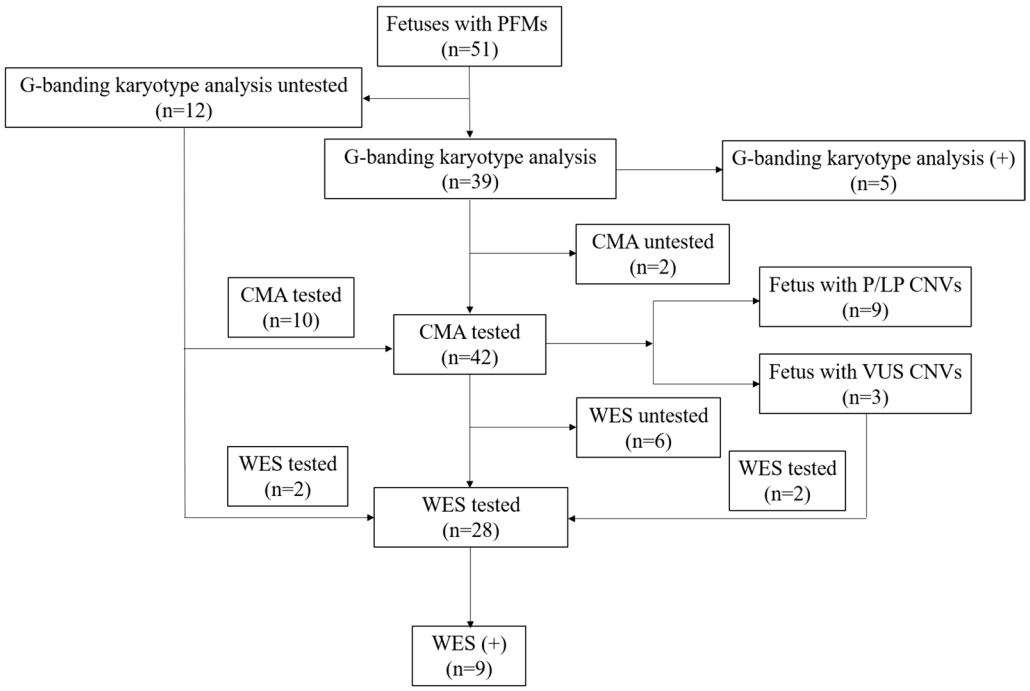

Fig. 1 Study profile. *PFM* posterior fossa malformation, *CNV* copy number variant, *WES* whole-exome sequencing, *P/LP* pathogenic/likely pathogenic, *VUS* variants of unknown significance

planes), sagittal (midsagittal anterior, midsagittal posterior, and parasagittal planes), and coronal planes (transfrontal, transcaudate, and transthalamic planes and a cross-section of the vermis) [10]. When PFMs were suspected during routine ultrasonographic screening (partial or complete absence of the vermis, enlarged posterior fossa, transverse diameter of the cerebellum small for gestational age, cyst in fetal posterior fossa), neurosonography was performed for further examination of the suspected PFMs [11]. To improve the diagnostic rate, fetal MRI was performed at an appropriate gestational age. T2-weighted ultrafast single-shot sequences were the main sequences used in fetal MRI. The axial, coronal, and sagittal planes of the fetal brain were obtained [12]. The diagnostic gold standard was determined using MRI or autopsy (Fig. 2).

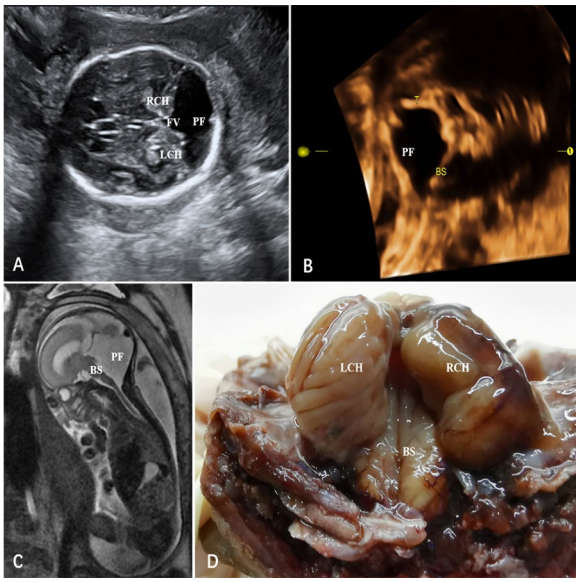

Fig. 2 Neuroimaging and autopsy of DWM. **A** Axial plane of 2D ultrasound: enlargement of the fourth ventricle and posterior fossa. **B** 3D ultrasound: vermis agenesis, enlargement of the fourth ventricle and posterior fossa, and elevated tentorium. **C** Sagittal plane of MRI: vermis agenesis, enlargement of the fourth ventricle and posterior fossa, and elevated tentorium. **D** The appearance of autopsy: vermis agenesis and left cerebellar hemisphere dysplasia. *LCH* left cerebellar hemisphere, *RCH* right cerebellar hemisphere, *FV* fourth ventricle, *PF* posterior fossa, *BS* brainstem, *T* tentorium of cerebellum, *DWM* Dandy–Walker malformation

Genetic testing

G-banding karyotyping was performed at a level of 300 bands according to the standardization process [13]. CNV testing includes CMA and low-pass WGS. CMA was performed with CytoScan 750K array (Affymetrix, Santa Clara, CA, USA) or the SurePrint G3 Human 8×60K microarray (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's recommendations. Low-pass WGS-based CNV testing was performed with unique reads ≥ 2.5 Mb on the Next Seq CN 500

platform (Beijing, China). CNVs were identified based on the human reference genome 37 (NCBI37) of the National Centre for Biotechnology Information. CNVs were classified into five types according to the American College of Medical Genetics guidelines (ACMG): pathogenic (P), likely pathogenic (LP), variants of unknown significance (VUS), benign, and likely benign [14]. WES trio (parents-fetus) was performed using the whole-exome capture chip xGen Exome Research Panel v2.0 (Integrated DNA Technologies, Iowa, USA). The genetic data were analyzed through bioinformatics and clinical information analysis. Suspected pathogenic variants were validated by Sanger sequencing. Variants were classified into five types according to ACMG: P, LP, VUS, benign, and likely benign [15].

Statistical analyses

Continuous variables were expressed as mean ± standard deviation. SPSS version 23.0 (IBM Corp., Armonk, NY, USA) was used for the statistical data analysis.

Results

General data of patients with PFMs

Based on the results of prenatal ultrasound, MRI, and autopsy, 51 fetuses with PFMs were analyzed, including 11 with isolated and 40 with non-isolated PFMs. Among the 40 non-isolated fetuses, 13 had additional intracranial anomalies only and 27 had both intracranial and extracranial anomalies. Congenital heart defects (42.50%; 17/40) and corpus callosum malformations (40.00%; 16/40) were the most and second most common complications, respectively. The different phenotypes of the PFMs are shown in Table S1.

Genetic data of isolated PFMs

G-banding karyotype analysis was successfully performed in nine participants with isolated PFMs, and all showed negative results. CNV testing was performed in 11 cases, revealing four cases with P/LP CNVs, corresponding to a detection rate of 36.36%, as shown in Table 1. All the CNVs were de novo. Of the remaining seven cases with negative CNV findings, six cases were analyzed using WES; all cases showed negative results. All the participants with P/LP CNVs chose pregnancy termination.

Genetic data of non-isolated PFMs

G-banding karyotype analysis was successfully performed for 30 participants with non-isolated PFMs. Five of these participants had chromosomal abnormalities, including one case of trisomy 18, two cases of trisomy 13, one case of 45,X, and one case of 47,XN, + del(22)(q13), as shown in Table 2. The detection rate of G-banding karyotyping was 16.67% (5/30). These five patients opted to terminate their pregnancies.

CNV testing was performed in 31 fetuses with non-isolated PFMs; P/LP CNVs were detected in five cases. All the CNVs were de novo. The diagnostic rate of CNV testing was 16.13% (5/31), as shown in Table 3. All of the patients with P/LP CNVs chose pregnancy termination. Twenty-two cases with non-isolated PFMs were analyzed using WES; nine of them had P/LP variants, with an overall diagnostic rate of 40.91% (9/22). Out of the nine

Table 2 Non-isolated PFMs of abnormal G-banding karyotype analysis

Case	Phenotype	Result	Outcome
5	DWM, AVSD	47,XN, + del(22)(q13)	TOP
6	DWM, TOF, SUA	Trisomy 13	TOP
7	VH, MCM, ACC, VSD, strephenopodia	Trisomy 18	TOP
8	VH, PA, VSD, RAA, strephenopodia	Trisomy 13	TOP
9	DWM, AS	45,X	TOP

DWM Dandy–Walker malformation, *AVSD* atrioventricular septal defect, *TOF* tetralogy of Fallot, *SUA* single umbilical artery, *VH* vermis hypoplasia, *MCM* mega cisterna magna, *ACC* agenesis of the corpus callosum, *VSD* ventricular septal defect, *PA* pulmonary atresia, *RAA* right aortic arch, *TOP* termination of pregnancy

Table 1 Characteristics of different CNVs in fetuses with isolated PFMs

Case	Phenotype	CNVs	Size	Classification	Known syndrome	Genes involved	Outcome
1	DWM	seq[GRCh37] dup(8)(p11.21p11.1) NC_000008.10:g.4088000_43800000dup	2.92 Mb	P	–	*POMK*	TOP
2	VH	arr[GRCh37] 3p26.3p22.1(61891_41337636) × 3, 9p24.3p24.1(322793_8236122) × 1	41.2 Mb 7.9 Mb	P	–	*ITPR1 DOCK8, DMRT1*	TOP
3	DWM	seq[GRCh37] dup(7)(p22.2p21.2) NC_000007.13:g.3720000_15180000dup	11.46 Mb	LP	–	–	TOP
4	DWM; AC	seq[GRCh37] del(6)(q24.1q24.3) NC_000006.11:g.141849894_145690389del	3.84 Mb	P	MRD43	*HIVEP2*	TOP

CNV copy number variation, *DWM* Dandy–Walker malformation, *VH* vermis dysplasia, *AC* arachnoid cyst, *MRD43* mental retardation autosomal dominant 43, *P* pathogenic, *LP* likely pathogenic, *TOP* termination of pregnancy

Table 3 Characteristics of different CNVs in the fetuses with non-isolated PFMs

Case	Phenotype	CNVs	Size	Classification	Known syndrome	Genes involved	Outcome
10	BPC; LVD	seq[GRCh37] dup(9)(p24.3p13.1) NC_000009.11:g.20000_58780000dup	38.58 Mb	P	–	–	TOP
11	CH; SUA	seq[GRCh37] del(5)(p15.33p13.3) NC_000005.9:g.20000_30120000del	30.10 Mb	P	Cri du chat syndrome	*TRIO*	TOP
12	VH; MCM; TOF; DCC; polyhydramnios	seq[GRCh37] del(22)(q11.21q11.21) NC_000022.10:g.18888282 5_21796237del	2.91 Mb	P	DiGeorge syndrome	*TBX1*	TOP
13	BPC; ACC	seq[GRCh37] del(X)(p11.23p22.33), del(X)(q13.2q28) NC_000023.10:g.[2699472_47700577del];[72764487_154873016del]	45.00 Mb 82.11 Mb	P	Ullrich–Turner syndrome	*POF1B, BHLHB9, DACH2, DIAPH2, CENPI, PGRMC1, BCORL 1, XPNPEP2, FMR1, FMR2/AFF2*	TOP
14	CH; ACC; cortical hypoplasia	seq[GRCh37] del(6)(q25.3q27), dup(7)(q36.3) NC_000006.11:g.160780000_170920000del NC_000007.13:g.155300000_159138663dup	10.14 Mb 3.84 Mb	P	–	*DLL1, LMBR1*	TOP

CNV copy number variation, *DWM* Dandy–Walker malformation, *VH* vermis dysplasia, *BPC* Blake's pouch cyst, *LVD* lateral ventricle dilation, *CH* cerebellar hypoplasia, *SUA* single umbilical artery, *MCM* mega cisterna magna, *TOF* tetralogy of Fallot, *DCC* dysplasia of corpus callosum, *ACC* agenesis of corpus callosum, *P* pathogenic, *LP* likely pathogenic, *TOP* termination of pregnancy

cases, variants in *CEP20* or *TMEM67* were identified in five cases, and *OFD1*, *PTPN11*, *AR1DIA*, and *SMARCA4* variants were found in four cases of Oral–Facial–Digital type I syndrome, Noonan syndrome, Coffin–Siris syndrome 2, and Coffin–Siris syndrome 4, respectively (Table 4).

Stratified analysis result of PFMs

As shown in Table 5, 23 abnormal genetic results were detected using conventional karyotyping, CNV testing, and WES, with an overall detection rate of 45.10% (23/51). The overall detection rates of karyotyping, CNV testing and WES of isolated PFMs were 0.00%, 36.36%, and 0.00%, respectively. In non-isolated PFMs, karyotyping, CNV testing and WES showed overall detection rates of 16.67%, 16.13%, and 40.91%, respectively. Furthermore, regarding the different phenotype of PFMs, the overall detection rates of DWM, JS, BPC, VH, CH, and RES were 41.18% (7/17), 83.33% (5/6), 18.18% (2/11), 77.78% (7/9), 28.57% (2/7), and 0.00% (0/1), respectively.

Outcomes

Of the 51 fetuses enrolled in the study, only six were born (five BPC cases and one case of unilateral cerebellar hemisphere dysplasia). In these six fetuses, normal results were obtained in genetic diagnosis; furthermore, three fetuses with isolated BPC and one fetus with unilateral cerebellar hemisphere dysplasia were well developed during follow-up at 4, 5, 17, and 36 months, respectively. However, one newborn with BPC presented with postnatal polydactyly and syndactyly. At 4 months, brain MRI indicated kernicterus, and the child presented with moderate-to-severe growth retardation and neurodevelopmental retardation at follow-up. Another child with non-isolated BPC, which is associated with corpus callosum dysplasia, was in a "borderline state" at the age of 2.5 years.

Discussion

Main findings

PFM is associated with multiple genetic factors. Different genetic technologies provide new perspectives for the prenatal diagnosis of PFM. In our cohort, we conclude an optimal prenatal genetic diagnostic approach for PFMs. Considering cost-effectiveness, we recommend CNV testing as the primary genetic diagnostic method for isolated PFMs. A sequential genetic approach (karyotyping, CNV testing, and WES) should be performed in non-isolated PFMs. When the results of karyotyping and CNV testing are negative, additional WES testing may be required to further analyze the genetic etiology. In

Table 4 Characteristics of WES and clinical phenotypes of patients with non-isolated PFMs

Case	Phenotype	Gene	Classification	Location	Variation	Isoform	Mode of inheritance	Genotype	Outcome
15	JS	*CEP290*	P LP	chr12:88478620–88478623 chr12:88523518	c.4445_c.4448delAAGA c.806delA	NM_025114 NM_025114	AR	het	TOP
16	JS	*CEP290*	P P	chr12:88471628–88471629 chr12:88462434	c.5434_c.5435delGA c.6012-12 T > A	NM_025114 NM_025114	AR	het het	TOP
17	JS	*TMEM67*	P	Chr8:94768099	c.312 + 5G > A	NM_153704	AR	hom	TOP
18	JS	*CEP290*	P P	chr12:88462424–88462424 chr12:88481689–88481689	c.6012-2A > G c.4062delT	NM_025114.3 NM_025114.3	AR	het het	TOP
19	JS	*TMEM67*	P	chr8:94797493	c.1175C > G	NM_153704	AR	hom	TOP
20	VH; HCM; AS	*PTPN11*	LP	Chr12:112489093	c. 1517 A > C	NM_002834	AD	het	TOP
21	VH; ACC; VSD; FGR	*SMARCA4*	LP	Chr19:11134230	c.2896C > T	NM_001128849	AD	het	TOP
22	VH; cortical hypoplasia; schizencephaly; ACC; CPC	*OFD1*	LP	chrX:13775868	c.1081G > T	NM_001330210	X-linked	het	TOP
23	DWM; ACC; VSD; PLSVA	*ARID1A*	LP	Chr1:27107105	c.6716T > C	NM_006015	AD	het	TOP

WES whole-exome sequencing, *JS* Joubert syndrome, *VH* vermis dysplasia, *HCM* hypertrophic cardiomyopathy, *AS* aortic stenosis, *ACC* agenesis of corpus callosum, *VSD* ventricular septal defect, *FGR* fetal growth restriction, *CPC* choroid plexus cysts, *DWM* Dandy–Walker malformation, *PLSVC* persistent left superior vena cava, *P* pathology, *LP* likely pathology, *VUS* variants of unknown significance, *AR* autosomal recessive inheritance, *AD* autosomal dominant inheritance, *TOP* termination of pregnancy

addition, we recommend WES as a first-tier genetic diagnostic tool when JS is suspected.

Interpretation of findings

In isolated PFMs, our present detection rate of CNV testing (36.36%) is much higher than that of karyotyping and WES (both were 0.00%). Thus, we recommend CNV testing as the first-line cytogenetic diagnostic test for isolated PFMs. It is noteworthy that advancing gestation (> 24 weeks) is associated with a significant increase in the laboratory failure rate of karyotyping, especially in the third trimester. In this situation, CNV testing, instead of karyotyping analysis, can provide valuable genetic information, as some cases are diagnosed too late (for example, Cases 2, 10, 11, 13, and 14). We believe that WES is of limited value in the genetic diagnosis of isolated PFMs, although WES has been used in multiple structural abnormalities to provide a higher resolution in the identification of monogenic disorders. Two previous studies support our position. Tan's study [16] reported that two fetuses with isolated PFM (one case of isolated DWM and one case of isolated BPC) had negative results with the use of WES. Another study recruited 268 fetuses with central nervous system malformations, and seven fetuses with isolated PFM (five cases with DWM and two cases with CH) had a negative genetic work-up based on WES [17]. However, Li et al. [18] studied seven patients with isolated cerebellar vermis defects and found two cases of DWM with a single gene defect, with a detection rate of 28.57%. However, one of the patients was found to have a VOUS, which should be excluded from the positive results. Besides, most of the patients in the study underwent proband-only WES, making the results difficult to verify. Therefore, the high detection rate has limited credibility. Given the high cost of WES, the low detection rate of P/LP variants, and the difficulty of variant analysis, we recommend that when CNV testing presents negative findings in isolated PFMs, WES may be not considered as a routine screening tool for isolated PFMs.

In non-isolated PFMs, we recommend a sequential genetic approach (karyotyping, CNV testing, and WES) for genetic diagnosis. Considering the cost-effectiveness, the sequential genetic approach was applied in our study; the result showed a high detection rate of 47.5% (19/40) of the genetic abnormalities. Compared to its application in isolated PFMs, WES can provide additional diagnostic value in non-isolated PFMs (0.00% vs. 40.91%). In a study of 34 cases of PFMs with multisystem organ abnormalities, Drexler et al. [19] estimated a diagnostic yield of over 50% using WES. Besides, the role of WES in the genetic diagnosis of PFMs associated with other malformations has also been demonstrated in a number of case reports [20–22]. We have shown that WES may provide

Table 5 The detection rate of different types of PFMs

Type	Total	G-banding karyotype analysis			CNV testing			WES		
		Total	Isolated	Non-isolated	Total	Isolated	Non-isolated	Total	Isolated	Non-isolated
DWM	7/17 (41.18%)	3/14 (21.43%)	0/5 (0.00%)	3/9 (33.33%)	3/13 (23.08%)	3/6 (50.00%)	0/7 (0.00%)	1/11 (9.09%)	0/3 (0.00%)	1/8 (12.5%)
VH	7/9 (77.78%)	2/7 (28.57%)	0/0 (0.00%)	2/7 (28.57%)	2/8 (25.00%)	1/1 (100%)	1/7 (14.29%)	3/5 (60.00%)	0/0 (0.00%)	3/5 (60.00%)
CH	2/7 (28.57%)	0/3 (0.00%)	0/1 (0.00%)	0/2 (0.00%)	2/5 (40.00%)	0/1 (0.00%)	2/4 (50.00%)	0/2 (0.00%)	0/1 (0.00%)	0/1 (0.00%)
BPC	2/11 (18.18%)	0/9 (0.00%)	0/3 (0.00%)	0/6 (0.00%)	2/10 (20%)	0/3 (0.00%)	2/7 (28.570%)	0/4 (0.00%)	0/2 (0.00%)	0/2 (0.00%)
JS	5/6 (83.33%)	0/5 (0.00%)	0/0 (0.00%)	0/5 (0.00%)	0/5 (0.00%)	0/0 (0.00%)	0/5 (0.00%)	5/6 (83.33%)	0/0 (0.00%)	5/6 (83.33%)
RES	0/1 (0.00%)	0/1 (0.00%)	0/0 (0.00%)	0/1 (0.00%)	0/1 (0.00%)	0/0 (0.00%)	0/1 (0.00%)	0/1 (0.00%)	0/0 (0.00%)	0/1 (0.00%)
Total	23/51 (45.10%)	5/39 (12.82%)	0/9(0.00%)	5/30(16.67%)	9/42 (21.43%)	4/11 (36.36%)	5/31 (16.13%)	9/28 (32.14%)	0/6 (0.00%)	9/22 (40.91%)

PFM posterior fossa malformation, *CMA* chromosomal microarray analysis, *WES* whole-exome sequencing, *DWM* Dandy–Walker malformation, *VH* vermis dysplasia, *CH* cerebellar hypoplasia, *BPC* Blake's pouch cyst, *JS* Joubert syndrome, *RES* rhombencephalosynapsis

additional diagnostic value in non-isolated PFMs. Therefore, a sequential genetic approach is the most appropriate diagnostic strategy for non-isolated PFMs.

In addition, our study revealed that a large percentage of our JS cases (5/6, 83.33%) had positive genetic variant using WES, which is consistent with the result that pathogenic variants in the associated genes account for ~ 60–90% of JS cases [23]. Based on our data, WES can be considered a first-tier genetic diagnostic approach for JS. Moreover, previous studies have demonstrated that WES can evaluate genotype–phenotype correlations in JS. The "molar tooth sign", represented by the combination of CH, abnormally thick and horizontally oriented superior cerebellar peduncles, and/or a deep interpeduncular fossa, is a typical sign of JS [24]. When "molar tooth sign" is suspected, WES can be used directly as a first-line diagnostic tool instead of the sequential approach (karyotyping to CNV testing, and then WES) to save medical costs and improve the cost–benefit ratio.

Notably, three fetuses with isolated BPC in our study had negative genetic findings using prenatal sequential genetic testing (karyotyping, CNV testing, and WES) and were born with good outcomes, consistent with other studies [7, 8, 25]. A meta-analysis showed a very low rate of chromosomal anomalies (5.2%) in cases of isolated BPC [7, 8]. However, there is no study on the value of WES in cases of isolated BPC. Therefore, based on our data, we do not recommend routine use of WES in individuals with isolated BPC. However, the diagnostic strategy for non-isolated BPC remains cautious. It depends on the type and severity of associated malformations; for example, Case 10 and 13 had P CNVs with poor prognosis.

Before applying different strategies for the diagnosis of isolated and non-isolated PFMs, accurate prenatal diagnosis of PFMs using ultrasound is important. Our study revealed additional fetal anomalies in PFMs, including congenital heart defects (CHDs), corpus callosum malformations, and cortical hypoplasia. CHDs were the most common type of extracranial anomaly. Trisomy 13, Trisomy 18, Turner syndrome, 22q11 deletion syndrome, Noonan syndrome, Coffin–Siris syndrome 2, and Coffin–Siris syndrome 4 were observed in Cases 5, 6, 7, 8, 9, 12, 20, 21, and 23. Phenotypes of DWM, small cerebellar diameter, abnormal vermis, and clivus heights were common in these genetic syndromes [26–31]. The reason PFMs are often associated with CHD can be explained by two hypotheses. First, affected signals or pathways in aneuploidy or genetic syndromes may explain the mechanism underlying the co-occurrence of cardiac malformations and PFMs, such as Noonan syndrome [32–34]. Second, some studies have suggested that CHD exerts devastating effects on neurological development via blood circulation [32–34]. Therefore, once PFMs are detected, there should be a focus on the cardiovascular system to prevent misdiagnosis.

Strengths and limitations

Our study has several strengths. First, our study is the first relatively large study to focus on prenatal genetic diagnostic strategy for PFMs. Second, our study first recommended optimal prenatal genetic diagnostic strategies for isolated and non-isolated PFMs. Third, our study recommends WES as a first-tier genetic diagnostic tool when JS is suspected.

Our study also has some limitations. Some cases were diagnosed > 24 weeks with failed karyotyping and some patients refused complete genetic testing, leading to the incomplete data. In addition, our conclusion on the diagnostic strategies for different subtypes of PFMs requires a prospective study with large sample size for verification and promotion.

Conclusions

Since chromosomal CNVs are the primary cause of isolated PFMs and the additional contribution of WES is not significant; we recommend CNV testing for pregnant women with isolated PFMs. A sequential genetic approach (karyotyping, CNV testing, and WES) can benefit pregnancies with non-isolated PFMs. Particularly, we recommend WES as the first-line diagnostic tool for JS.

Abbreviations

PFM	Posterior fossa malformation
CNV	Copy number variant
CMA	Chromosome microarray analysis
WES	Whole-exome sequencing
DWM	Dandy–Walker malformation
JS	Joubert syndrome
BPC	Blake's pouch cyst
VH	Vermian hypoplasia
CH	Cerebellar hypoplasia

Supplementary Information

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Supplementary Material 1.

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Author contributions

SLJ and WQQ conceived the study. GCX, CYJ, LY, YL, ZTJ, WL, HJJ, and ZGH performed fetal examinations. YYS carried out genetic data analysis. GCX and ZJ performed data extraction. ZJ drafted the first version of the paper, which was edited and approved by all the authors.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study has been approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital, Capital Medical University (Approval No. 2017-KY-076-01).

Competing interests
The authors declare no competing interests.

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