

RESEARCH

Open Access



Molecular diagnostic yield of exome sequencing in a Chinese cohort of 512 fetuses with anomalies

Pengzhen Jin^{1†}, Jiawei Hong^{1†}, Yuqing Xu¹, Yeqing Qian^{1,2}, Shuning Han¹ and Minyue Dong^{1,2*}

Abstract

Background Currently, whole exome sequencing has been performed as a helpful complement in the prenatal setting in case of fetal anomalies. However, data on its clinical utility remain limited in practice. Herein, we reported our data of fetal exome sequencing in a cohort of 512 trios to evaluate its diagnostic yield.

Methods In this retrospective cohort study, the couples performing prenatal exome sequencing were enrolled. Fetal phenotype was classified according to ultrasound and magnetic resonance imaging findings. Genetic variants were analyzed based on a phenotype-driven followed by genotype-driven approach in all trios.

Results A total of 97 diagnostic variants in 65 genes were identified in 69 fetuses, with an average detection rate of 13.48%. Skeletal and renal system were the most frequently affected organs referred for whole exome sequencing, with the highest diagnostic rates. Among them, short femur and kidney cyst were the most common phenotype. Fetal growth restriction was the most frequently observed phenotype with a low detection rate (4.3%). Exome sequencing had limited value in isolated increased nuchal translucency and chest anomalies.

Conclusions This study provides our data on the detection rate of whole exome sequencing in fetal anomalies in a large cohort. It contributes to the expanding of phenotypic and genotypic spectrum.

Keywords Fetal whole exome sequencing, Fetal anomalies, Recurrent phenotype, Frequent gene

Introduction

Fetal anomalies occur in approximately 4–6% pregnancies [1], which result in increased perinatal morbidity and mortality, causing economic and mental burden on the families and society [2]. Ultrasound scanning is a preferred method to identify fetal anomalies, ranging

from minor deformity to multiple anomalies [3, 4]. Some anomalies, such as ventricular septal defect and lymphatic hygroma, may have good prognoses, whereas, pathogenic genetic causes can yield contrary outcomes [5–7]. Therefore, genetic investigations are necessary in the assessment of fetal prognoses and clinical decision-making.

Common genetic causes including chromosomal abnormalities, copy number variations (CNVs) and single-nucleotide variants (SNVs) [8]. Generally, diagnostic yield for karyotyping is nearly 32% in fetuses with anomalies [9, 10]. Together with chromosomal microarray (CMA), detection rates can be increased by 3–10% [1, 3, 11]. Whole exome sequencing (WES) are recommended

[†]Pengzhen Jin and Jiawei Hong share first authorship.

*Correspondence:

Minyue Dong
dongmy@zju.edu.cn

¹Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, China

²Key Laboratory of Reproductive Genetics (Zhejiang University), Ministry of Education, Hangzhou, China



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

by the American College of Medical Genetics and Genomics (ACMG) (2020) as a helpful complement after negative findings in karyotyping and CMA [12]. Currently, publications about the application of fetal WES in prenatal diagnosis are increased, with a reported diagnostic yield varying from 6.1–44% [1, 3, 13–19]. Regrettably, only four cohorts are reported with relatively large sample size ($n > 500$) [1, 3, 13, 14]. Therefore, data on its clinical utility remain limited in practice.

Herein, we reported our data of fetal WES in a Chinese cohort of 512 trios to evaluate its detection value for fetal anomalies. Fetal phenotype was classified according to ultrasound and magnetic resonance imaging (MRI) findings and variants were analyzed with a phenotype-driven followed by genotype-driven strategy. This study will contribute to a more comprehensive understanding of the clinical efficiency of prenatal WES and expand the phenotypic and genotypic spectrum.

Methods

Study design and participants

It is a retrospective cohort study in a tertiary referral centre (Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, China), from November 1, 2021 to January 16, 2024. Clinical data were collected until April 23, 2024.

Invasive prenatal tests were performed in pregnant women whose fetuses manifesting as structural anomalies. The data interpretation was made during pregnancy, after a turnaround time of WES for nearly 2 weeks, and the couple might choose to continue or terminate the pregnancy, based on the WES results. In the current investigation, families in accordance with the following criteria were recruited: (1) fetal anomalies were diagnosed by a qualified physician with ultrasound scanning or MRI findings; (2) fetal WES was performed and peripheral blood for both parents were available for further WES analysis. Exclusion criteria were pregnancies with aneuploidies or pathogenic copy number variations. Genetic and phenotypic data, as well as the outcome of the pregnancy of the couple were collected and analyzed.

Written informed consents were obtained and the study was approved by the Ethics Committee of Women's Hospital, School of Medicine Zhejiang University (IRB-20240128-R).

DNA extraction

Fetal DNA was extracted from chorionic villi in three (0.59%) cases, amniotic fluid in 341 (66.6%), umbilical cord blood in 98 (19.14%) and tissue samples after miscarriage or termination of pregnancy (TOP) in 70 (13.67%). The average gestational age of villocentesis was 13.55 ± 0.51 weeks, amniocentesis, 22.54 ± 0.55 and trans-abdominal umbilical blood puncture, 29.55 ± 0.71 weeks.

DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The DNA concentrations were detected using the NanoDrop 2000 (Thermo Fisher Scientific, USA).

Whole exome sequencing

DNA samples from parents-fetus trios were sequenced on Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA), according to the manufacturer's instructions. The sequencing reads were referred to the Genome Reference Consortium Human genome build 37 (GRCh37) or humangenome 19 (hg19). Variants were filtered by population databases, including genome Aggregation Database (gnomAD) and Exome Aggregation Consortium (ExAC). Then, the variants were compared by multiple databases, including the Human Gene Mutation Database, OMIM, DECIPHER and ClinVar database. Only those with allele frequency $\leq 1\%$ would be retained. To predict the pathogenicity of the mutations, several softwares were used, including the Sorting Intolerant from Tolerant (SIFT), Mutation Taster and Splice AI. The variants were interpreted under the guideline of the ACMG. In case of variants of unknown significance, only those highly compatible with the phenotype or frequently identified in more than one family would be collected and analyzed. Then, Sanger sequencing was carried out to validate the variants. The products were sequenced with the ABI 3730 DNA analyzer (Applied Biosystems™) and analyzed by DNASTAR 5.0 software.

Results

Clinical characteristics of participants

A total of 512 fetuses with anomalies were recruited after excluding clinically relevant abnormal karyotyping or chromosomal microarray findings. 501 were singleton pregnancies and 11 were twin. The median age of the pregnant women was 30 years old (range, 22–43 years) and gestational age at the time of testing was 24 (range, 13–33 weeks). Parents opted for termination in 246 pregnancies (48.05%). 12 fetuses ended in intrauterine death (2.34%), 48 were premature birth (9.38%) and 206 were full-term birth (40.23%). Demographic characteristics and outcomes of the study were shown in Table 1.

Distribution of the fetal anomalies and its diagnosis rates

The included fetuses manifesting with variable anomalies were grouped according to the fetal phenotype. 180 fetuses with malformations involving at least two systems were classified as multisystem anomalies. 332 fetuses manifested with single-system malformations, involving 65 in skeletal, 49 in genito-urinary, 46 in central nervous systems, 44 in increased nuchal translucency (NT), 34 in cardiac, 33 in facial, 32 in fetal hydrops, 26 in abdominal and three in chest anomalies.

Table 1 Demographic characteristics and outcomes of the study cohort

Variable	Value
Maternal Age	30 (22–43)
Gestational age (weeks)	24 (13–33)
Singleton pregnancy	501 (97.9%)
Twin pregnancy	11 (2.1%)
Source of fetal DNA	
Chorionic villi	3 (0.59%)
Amniotic fluid	341 (66.6%)
Umbilical cord blood	98 (19.14%)
Tissue samples	70 (13.67%)
Pregnancy outcome	
Full-term birth (>=37 weeks)	206 (40.23%)
Premature birth (<37 weeks)	48 (9.38%)
Termination of pregnancy (TOP)	246 (48.05%)
Intrauterine death	12 (2.34%)

Table 2 Distribution of the fetal anomalies and its diagnosis rates

System	Number	Pathogenic number	Diagnosed rate
Skeletal	65	12	18.46%
Multisystem	180	31	17.22%
Genito-urinary	49	8	16.33%
Fetal hydrops	32	5	15.63%
Abdominal	26	3	11.54%
Central nervous	26	3	11.54%
Facial	33	3	9.09%
Cardiac	34	1	2.94%
Increased NT ^a	44	0	0%
Chest	3	0	0%
Overall	512	69	13.48%

*a: NT=nuchal translucency

Table 3 Frequently identified phenotype and its diagnosis rates

Phenotype	Number	Pathogenic number	Diagnosed rate
Short femur	39	11	28.21%
Ventriculomegaly	15	4	26.67%
Kidney cyst	18	4	22.22%
Cleft lip and/or palate	20	4	20.00%
Ventricular Septal Defect	24	3	12.50%
Varus foot	15	1	6.67%
Lymphatic hygroma	20	1	5.00%
Situs viscerum inversus	18	1	5.56%
Fetal growth restriction (FGR)	46	2	4.35%

As is shown in the Table 2, multisystem anomalies were frequently identified, with a diagnosis rate of approximately 17.22%. Among the single anomalies, skeletal system was the most frequently affected organ referred for prenatal WES, with a detection rate of 18.46%. The molecular diagnostic rate was relative low for fetuses with anomalies affecting facial and cardiac systems. None

Table 4 Features of the recurrent phenotype in the pedigree

Fetuses	Recurrent occurred phenotype	Pathogenic genes	Inheritance manner	Pathogenic variants	Inheritance
Case 1	Interventricular Septal Defect	<i>KMT2D</i>	AD	c.2263dup	de novo
Case 2	Short Limb	<i>RMRP</i>	AR	n.-35_-8dup n.5 C>T	Paternal Maternal
Case 3	Polyhydramnios	<i>MUSK</i>	AR	c.754-6T>A c.35T>C	Paternal Maternal
Case 4	Bowed and Short bones	<i>COL1A1</i>	AD	c.800del	Maternal
Case 5	Polydactyly, Encephalocele and Hydrocephalus, Ventriculomegaly	<i>OFD1</i>	XL	c.536T>C	Maternal
Case 6	Fetal Akinesia, Pleural Effusion	<i>MUSK</i>	AR	c.1870 C>T c.1928- 2 A>C	Paternal Maternal
Case 7	Renal Cyst	Not identified	/	/	/
Case 8	Hydrocephalus	<i>L1CAM</i>	XL	c.1098G>A	Maternal
Case 9	Lymphatic hygroma, fetal akinesia, arthrogyrposis	Not identified	/	/	/

of pathogenic variants were identified in fetuses present with isolated increased NT or chest anomalies.

Frequently identified phenotype and its diagnosis rates

In Table 3, nine phenotype were frequently identified in fetuses, including fetal growth restriction (FGR) in 46, short femur in 39, ventricular septal defect in 24, lymphatic hygroma in 20, cleft lip and/or palate in 20, kidney cyst in 18, situs viscerum inversus in 18, varus foot in 15 and ventriculomegaly in 15 cases. Among them, short femur, ventriculomegaly, kidney cyst and cleft lip and/or palate had relative high diagnosis rates. Pathogenic variants were rarely identified in lymphatic hygroma, situs viscerum inversus and varus foot.

Recurrent occurrence of the same phenotype in siblings

Fetus presenting similar structural anomalies with its siblings were collected in the Table 4. Most of the families failed to identify genetic causes in the previous conception. When the same fetal phenotype occurred in the second conception, the couple received invasive prenatal diagnosis. Recurrent occurred fetal phenotype included interventricular septal defect, short femur,

polyhydramnios, polydactyly, encephalocele, ventriculomegaly, fetal akinesia, pleural effusion, renal cyst, hydrocephalus, lymphatic hygroma and arthrogryposis.

Seven pathogenic variants were identified, six of which were inherited from the one or both of the parents. In case 1, interventricular septal defect was recurrently observed, but *KMT2D* was found to be de novo. In the case 4, bowed and short bones were frequently occurred, but the *COL1A1* variant was inherited from unaffected mother, with an autosomal dominant inheritance manner.

Identified candidate variants and data analysis

180 nucleotide mutations in 112 genes were identified as candidate variants. Finally, 97 diagnostic variants in 65 genes were identified in 69 fetuses after phenotype-driven strategy. Among them, 29 genes were identified in more than one fetus (Fig. 1).

Variable variants in *TSC2* ($n=5$), *FGFR3* ($n=4$), *MUSK* ($n=4$), *PKD1* ($n=3$), *COL2A1* ($n=3$), *COL1A1* ($n=2$), *HNF1B* ($n=2$) and *TCOF1* ($n=2$) exhibited highly similar phenotype. While fetuses with variants of *KMT2D* ($n=2$) and *PTEN* ($n=2$) present with different anomalies (Table 5).

Discussion

This study shares the clinical data of fetal WES in 512 trios presenting with structural anomalies, with an average detection rate of 13.48%. Skeletal system was the most frequently affected organ referred for prenatal WES, and short femur and kidney cyst were the most common phenotype. WES had limited value for isolated increased NT and chest anomalies.

WES was first applied in the research arena around 2011 [20]. Since then, the capture, sequencing and

analysis of gene ushered an advancement swiftly and vigorously. It is an efficient diagnostic technology to identify novel pathogenic variants based on careful and complete evaluation of correlations between genotype and phenotype. The idea of performing fetal WES was endorsed in ACMG, and it has recently been considered as a first-tier method in fetal anomalies [11, 21].

The application of WES in prenatal diagnosis can assist in determining fetal prognosis and help to relieve couples' anxiety to some extent, however, the ambiguous interpretations of variants of unknown significance may produce new anxiety [22]. As WES can generate a huge number of variants, the identification of specific phenotype in the proband is of vital significance. Regrettably, as is shown in Table 3, fetal anomalies observed in the ultrasound or MRI are more common in short limbs, large or small head circumference, polyhydramnios or oligohydramnios and so on, which are too ambiguous to guide the interpretation. In addition, some characteristics such as skin, intelligence, movement, language, hearing, smelling, touching and so on can not present in the fetal period. Therefore, the detection rate in fetal anomalies is significantly lower than that reported in children or adults, even with a similar sequencing coverage and interpretation strategy [22–24].

The application of fetal WES should grasp the indications strictly. Undoubtedly, the results of publications are strong evidence on the value of fetal WES in the clinical use. Investigations about the detection rate of fetal WES is reported every now and then. The earliest publication can be dated to 2018 [4], Normand et al. recruited 146 couples performing fetal WES from 2012 to 2017. An overall molecular diagnostic rate of 32% was concluded, which greatly promoted the application of fetal WES. Subsequently, two large cohorts were performed

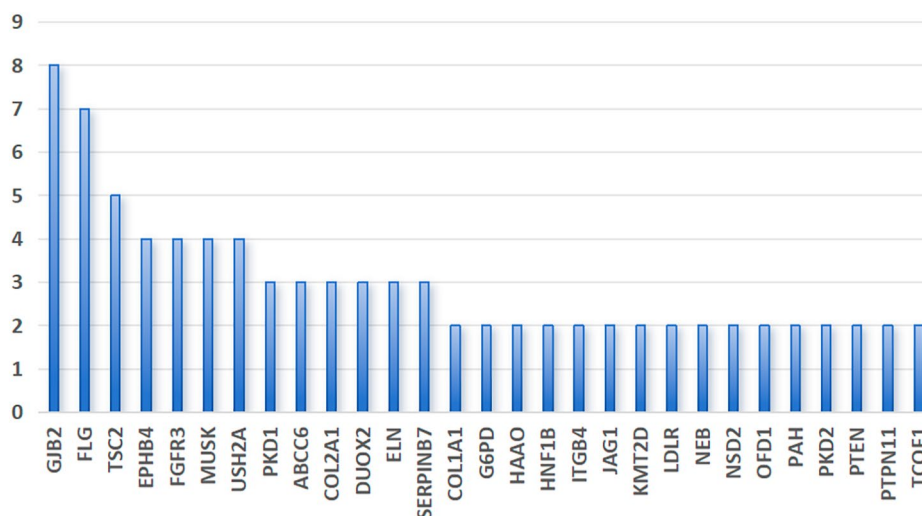


Fig. 1 Frequently identified genes in more than one fetus

Table 5 Features of the frequent genes and related fetal phenotype

Gene	Variant	Prenatal Ultrasound or MRI Imaging
TSC2	c.4289_4299delinsC	Cardiac rhabdomyomata with subependymal nodules
	deletion 9.12Kb	Cardiac rhabdomyomata with a slight widen of posterior corner in lateral ventricle
	c.4830G>A	Cardiac rhabdomyomata with subependymal nodules
	c.5228G>A	Cardiac rhabdomyomata with subependymal giant cell astrocytoma
	c.5238_5255del	Cardiac rhabdomyomata
FGFR3	c.742 C>T (N=2)	Bowed and short bones; Interventricular septal defect; Bell-shaped chest
	c.1138G>A (N=2)	Short limbs
MUSK	c.35T>C	Polyhydramnios (the amniotic fluid index is 45.6 cm)
	c.754-6T>A	Fetal akinesia and hydrops
	c.1928-2 A>C	
PKD1	c.1870 C>T	
	c.2180T>C	Increased renal parenchymal echogenicity; Echogenicity in left ventricular; Tricuspid regurgitation
	c.12,124 C>T	Polyhydramnios
COL2A1	c.10,420 C>T	Increased renal parenchymal echogenicity; Interventricular septal defect; Increased NT
	c.2710 C>T	Short limbs
	c.1628G>T	Short limbs; Narrow chest
COL1A1	c.1186G>C	Bowed and short bones; Polyhydramnios
	c.800del	Bowed bones
HNF1B	c.1128del	Bowed bones; Echogenicity in left ventricular
	c.544+3_544+6 del	Increased renal parenchymal echogenicity; Boundary between the cortex and medulla was clear; Polyhydramnios
	c.1339+5G>C	Increased renal parenchymal echogenicity; Enlarged kidneys; Boundary between the cortex and medulla was unclear
TCOF1	c.2604_2605del	Facial anomalies(Cleft palate; Micrognathia; Absence of external auditory meatus); Polyhydramnios
	c.2860G>T	Facial anomalies(Cleft Palate; Micrognathia; Widen of eye distance; Absence of nasal bone and external auditory meatus);
KMT2D	c.2263dup	Interventricular septal defect; Left ventricular hypoplasia with double outlet right ventricle
	c.16,018 C>T	Pleural effusion; Hydrops
PTEN	c.138 C>A	Microgyria; Subependymal nodules; A slight widen of posterior corner in lateral ventricle
	c.238 A>G	Macrocephaly; Microgyria

in 2019 [3, 13], with a rate of 8.5% and 10% respectively, enormously different from the previous conclusions. The amount, region and race discrepancy of subjects recruited might be the primary causes. Generally, detection rates will be largely overestimated in relative small cohorts [16].

The use of fetal WES is booming, with continuously evolving sequencing and analyzing technologies. Timely collection of clinical data and an accurate evaluation of detection value in large cohort is of vital significance to guide the application. Up to now, large Chinese cohort is still limited and data in different regions are valuable.

According to the statistics uncovered in our cohort, 69 fetuses were finally diagnosed, with an average detection rate of 13.48%. As for the single anomalies, the detection rate ranged from zero to 18.46%, which are conformed with the previous large cohort studies [1, 3, 13, 14]. In brief, more variants were found in fetuses diagnosed with renal anomalies. Pathogenic variants identified in cardiac, central nervous anomalies and FGR in our center were slightly less than previous reports. None of diagnosed variants were identified in isolated increased NT and chest anomalies in our clinic.

Lymphatic hygroma, situs viscerum inversus and varus foot, isolated or coupled with other anomalies, were frequently identified in prenatal ultrasound imaging, with relative low diagnostic rates. Pathogenic variants were frequently identified in fetuses manifested with short femur, ventriculomegaly, kidney cyst and cleft lip and/or palate. The anomalies, such as ventricular septal defect and cleft lip and/or palate, can be repaired after birth and may have a good prognosis. However, pathogenic genetic causes can yield contrary outcomes. Therefore, the identification of its genetic causes is of vital significance.

The recurrent phenotype is a strong indication for prenatal WES. In the current investigation, recurrent phenotype in consecutive siblings were observed in nine pedigrees, seven of which were finally diagnosed. Three diagnosed variants were inherited in an autosomal recessive manner and two were in a X-linked inheritance. In case 1, the de novo variant of *KMT2D* was inherited in an autosomal dominant manner, hinting a possible occurrence of germline mosaicism. In the case 4, bowed and short bones were frequently occurred, and the *COL1A1* variant was inherited from unaffected mother. It means that it might have a good pregnancy outcome. However, the pregnant woman chose to terminate the pregnancy.

In conclusion, the analysis of WES data in a retrospective cohort of 512 unselected pedigrees shows the detection rate of WES in fetal anomalies. With negative findings in karyotyping and CMA, WES can add additional 13.48% detection rate in fetuses with anomalies. It provides a more comprehensive understanding of

prenatal whole exome sequencing and expands phenotypic and genotypic spectrum.

Abbreviations

CNVs	Copy number variations
SNVs	Single-nucleotide variants
CMA	Chromosomal microarray
WES	Whole exome sequencing
ACMG	The American college of medical genetics and genomics
NT	Nuchal translucency
TOP	Termination of pregnancy
SIFT	The sorting intolerant from tolerant
FGR	Fetal growth restriction

Acknowledgements

We would like to thank our patients who were voluntary to donate fetal samples and peripheral blood samples.

Author contributions

MD contributed to the study conception and design. Material preparation, data collection and analysis were performed by PJ, JH, YX, YQ and SH. The first draft of the manuscript was written by PJ. All authors read and approved the final manuscript.

Funding

This work was supported by Zhejiang Provincial Natural Science Foundation of China (grant numbers LY22H110004), the National Natural Science Foundation of China (grant numbers 82171848) and the Key Projects Jointly Constructed by the Ministry and the Province of Zhejiang Medical and Health Science and Technology Project (grant numbers WKJ-ZJ-2127).

Data availability

The datasets supporting the conclusions of this article are available in the BIG Submission Portal repository (PRJCA026341) (<https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA026341>).

Declarations

Ethics approval and consent to participate

Informed consent was obtained from all individual participants included in the study and the study was approved by the Ethics Committee of Women's Hospital, School of Medicine Zhejiang University (IRB-20240128-R).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 15 May 2024 / Accepted: 26 August 2024

Published online: 09 September 2024

References

- Chen X, Jiang Y, Chen R, Qi Q, Zhang X, Zhao S, Liu C, Wang W, Li Y, Sun G, et al. Clinical efficiency of simultaneous CNV-seq and whole-exome sequencing for testing fetal structural anomalies. *J TRANSL MED*. 2022;20(1):10.
- Quinlan-Jones E, Lord J, Williams D, Hamilton S, Marton T, Eberhardt RY, Rinck G, Prigmore E, Keelagher R, McMullan DJ, et al. Molecular autopsy by trio exome sequencing (ES) and postmortem examination in fetuses and neonates with prenatally identified structural anomalies. *GENET MED*. 2019;21(5):1065–73.
- Lord J, McMullan DJ, Eberhardt RY, Rinck G, Hamilton SJ, Quinlan-Jones E, Prigmore E, Keelagher R, Best SK, Carey GK, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. *Lancet*. 2019;393(10173):747–57.
- Fu F, Li R, Li Y, Nie ZQ, Lei T, Wang D, Yang X, Han J, Pan M, Zhen L, et al. Whole exome sequencing as a diagnostic adjunct to clinical testing in fetuses with structural abnormalities. *Ultrasound Obstet Gynecol*. 2018;51(4):493–502.
- Banka S, Metcalfe K, Clayton-Smith J. Trisomy 18 mosaicism: report of two cases. *WORLD J PEDIATR*. 2013;9(2):179–81.
- Hirono K, Hata Y, Ibuki K, Yoshimura N. Familial Ebstein's anomaly, left ventricular noncompaction, and ventricular septal defect associated with an MYH7 mutation. *J Thorac Cardiovasc Surg*. 2014;148(5):e223–6.
- Schlüter G, Steckel M, Schiffmann H, Harms K, Viereck V, Emons G, Burfeind P, Pauer HU. Prenatal DNA diagnosis of Noonan syndrome in a fetus with massive hygroma colli, pleural effusion and ascites. *Prenat Diagn*. 2005;25(7):574–6.
- Pei J, Grishin NV. The DBSAV database: Predicting Deleteriousness of single amino acid variations in the human proteome. *J MOL BIOL*. 2021;433(11):166915.
- Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med*. 2012;367(23):2175–84.
- Hillman SC, Pretlove S, Coomarasamy A, McMullan DJ, Davison EV, Maher ER, Kilby MD. Additional information from array comparative genomic hybridization technology over conventional karyotyping in prenatal diagnosis: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2011;37(1):6–14.
- Yates CL, Monaghan KG, Copenheaver D, Retterer K, Scuffins J, Kucera CR, Friedman B, Richard G, Juusola J. Whole-exome sequencing on deceased fetuses with ultrasound anomalies: expanding our knowledge of genetic disease during fetal development. *GENET MED*. 2017;19(10):1171–8.
- Monaghan KG, Leach NT, Pekarek D, Prasad P, Rose NC. The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). *GENET MED*. 2020;22(4):675–80.
- Petrovski S, Aggarwal V, Giordano JL, Stosic M, Wou K, Bier L, Spiegel E, Brennan K, Stong N, Jobanputra V, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. *Lancet*. 2019;393(10173):758–67.
- Fu F, Li R, Yu Q, Wang D, Deng Q, Li L, Lei T, Chen G, Nie Z, Yang X, et al. Application of exome sequencing for prenatal diagnosis of fetal structural anomalies: clinical experience and lessons learned from a cohort of 1618 fetuses. *GENOME MED*. 2022;14(1):123.
- Mellis R, Oprych K, Scotchman E, Hill M, Chitty LS. Diagnostic yield of exome sequencing for prenatal diagnosis of fetal structural anomalies: a systematic review and meta-analysis. *Prenat Diagn*. 2022;42(6):662–85.
- Tran MF, Delanne J, Denommé-Pichon AS, Safrano H, Bruel AL, Vitobello A, Garde A, Nambot S, Bourgon N, Racine C, et al. Prenatal diagnosis by trio exome sequencing in fetuses with ultrasound anomalies: a powerful diagnostic tool. *FRONT GENET*. 2023;14:1099995.
- Vora NL, Norton ME. Prenatal exome and genome sequencing for fetal structural abnormalities. *AM J OBSTET GYNECOL*. 2023;228(2):140–9.
- Slavotinek A, Rego S, Sahin-Hodoglugil N, Kvale M, Lianoglou B, Yip T, Hoban H, Outram S, Anguiano B, Chen F, et al. Diagnostic yield of pediatric and prenatal exome sequencing in a diverse population. *NPJ GENOM MED*. 2023;8(1):10.
- Pauta M, Martinez-Portilla RJ, Borrell A. Diagnostic yield of exome sequencing in fetuses with multisystem malformations: systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2022;59(6):715–22.
- Bertier G, Sénécal K, Borry P, Vears DF. Unsolved challenges in pediatric whole-exome sequencing: a literature analysis. *Crit Rev Clin Lab Sci*. 2017;54(2):134–42.
- Lin XM, Li DZ. Prenatal genetic evaluation of fetuses with structural anomaly: is it time to shift from microarray to exome sequencing as a first-tier test? *Ultrasound Obstet Gynecol*. 2023;61(1):119–20.
- Janicki E, De Rademaeker M, Meunier C, Boeckx N, Blaumeiser B, Janssens K. Implementation of Exome sequencing in prenatal Diagnostics: chances and challenges. *Diagnostics (Basel)* 2023, 13(5).
- Lee H, Deignan JL, Dorrani N, Strom SP, Kantarci S, Quintero-Rivera F, Das K, Toy T, Harry B, Yourshaw M, et al. Clinical exome sequencing for genetic identification of rare mendelian disorders. *JAMA*. 2014;312(18):1880–7.
- Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, Braxton A, Beuten J, Xia F, Niu Z, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med*. 2013;369(16):1502–11.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.