Clinical utility of whole exome sequencing in the prenatal diagnosis with fetuses at risk

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Abstract

Purpose: To investigate the clinical utility of whole exome sequencing (WES) in the prenatal diagnosis with fetuses at risk and variation information identified by WES. Methods: WES was conducted for 40 families with clinical informed consent, and the overall diagnosis rate and performance in different subgroups were analyzed. Genetic variants identified by ES were assessed according to the Mendelian model of inheritance. Results: Of the 40 prenatal specimens, there were 28 cases of amniotic fluid, 6 cases of villi and 6 cases of fetal tissues. And the average gestational age was 19.5±4.8 weeks. It revealed that a total of 8 pathogenic / likely pathogenic variants were detected which likely to be associated with the observed fetal anomalies, and the diagnosis rate was 20% (8/40). Among them, the detection rate of fetal ultrasound abnormal group could be up to 60% (6/10). All 8 diagnosed cases had inherited the relevant variants (5 variants were autosomal recessively inherited and 3 were dominantly inherited disorders). Conclusion: The application of whole exome sequencing to prenatal diagnosis can improve the clinical diagnosis rate. WES has the potential to improve the clinical management of pregnancies and provide risk of recurrence in future pregnancies. However, further investigation was needed to maximize clinical usefulness of prenatal WES in the future.

Tweetable abstract

Prenatal WES has the potential to improve the clinical management of pregnancies and provide risk of recurrence in future pregnancies.

Keywords : whole exome sequencing, prenatal diagnosis, fetal structural anomaly, genetic variants

Introduction

About 70% of major birth defects are caused by genetic factors. In China, the incidence of newborns with severe genetic diseases exceeds 2%, and the total number exceeds 335,000. In addition to polygenic diseases, these serious genetic diseases include chromosomal diseases (˜1.2%), genomic diseases (Microdeletion/Microduplication Syndromes˜0.5%) and severe monogenic disease (˜0.5%). The powerful measure of preventing and controlling serious genetic diseases is prenatal diagnosis which will provide parents with pregnancy selection, decision-making information, recurrence risk counseling and pre-implantation genetic diagnosis options. At present, prenatal diagnostic techniques adopted clinically include first-generation sequencing, karyotyping, FISH, CMA, CNV-seq, WES and so on. The second-generation sequencing technology is rapidly popularized in the clinic, especially CNV-seq and WES techniques. Despite the comprehensive detection rate of karyotype analysis, FISH and CMA/CNV-seq reached about 40%, most of the genetic diseases are still difficult to be diagnosed.

Whole exome sequencing refers to a genomic analysis method that applies target capture technology to capture and enrich the whole genome exon region for high-throughput sequencing. Its application includes detecting SNV, indel (within 50 bp), exon level CNV and CNV above 100 kb. In addition, LOH can also be

detected by trio-WES. While there have been multiple publications showing a valuable diagnostic option of WES in postnatal genetic evaluation, integration of WES into prenatal diagnosis has been more limited[1]. Small-scale studies have confirmed that the diagnosis rate of WES in fetuses with structural abnormalities that were cytogenetically normal can reach 10%-57%. In 2019, two lancet studies suggested that the application of WES to prenatal diagnosis can improve the clinical diagnosis rate. In cases of fetal anomalies in which assessment with karyotype testing and chromosomal microarray fail to determine the underlying cause of a structural anomaly, WES can add clinically relevant information that could assist current management of a pregnancy [2,3]. In 2018, the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) came to an Joint Position Statement on the use of genome-wide sequencing for fetal diagnosis including whole exome sequencing, targeted analysis using clinical panels, and whole genome sequencing, which becomes the first guideline for clinical utility of next-generation sequencing in the prenatal diagnosis[4].

Herein, the aim of this study was to evaluate the clinical utility of whole exome sequencing in the prenatal diagnosis with fetuses at risk. We present a retrospective analysis of the outcomes of prenatal WES on 40 families conducted at a single prenatal genetic center. The diagnostic rate and additional information about genetic variants would help to facilitate the clinical utility of prenatal WES.

MATERIALS AND METHODS

Subjects

Research ethics board approval was obtained from the Human Ethics Committee of the first affiliated hospital of Zhengzhou university, and informed consents were gained all recruited subjects were signed before prenatal diagnosis were conducted. 40 families were recruited between May 2019 and September 2019. Detailed information including maternal age, gestational weeks, family history and clinical manifestation was documented. Parental peripheral blood and fetal samples including amniotic fluid, villi and induced fetal tissues were obtained by routine methods.

Whole exome sequencing

Genomic DNA samples were extracted from chorionic villi, amniocytes, fetal tissues or parental blood using a Qiagen DNA Blood Midi/Mini kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol. Then WES was performed according to the published paper[5]. And data was analyzed on the Cruxome system developed by Berry Genomics.

Sanger sequencing confirmation

All significant variants identified by WES were confirmed by PCR amplification and sanger sequencing as described in the published paper[6].

RESULTS

A total of 40 fetuses at risk were recruited to undergo WES detection including 39 trio-WES, 1 proband+trio WES. The average gestational age was 19.5±4.8 weeks. 3 different types of samples were detected. And amniotic fluid, villi and induced fetal tissues accounted for 70%(28/40), 15%(15/40), 15%(15/40)of all cases, respectively. Five fetuses were found to have significant pathogenic variants, and likely pathogenic variants were detected in 3 fetuses. All diagnosed cases including four carriers of autosomal recessive(AR)variants were summarized in table 1. All of the unselected fetuses were divided into five groups (table 2), and we found that clinical diagnosis rate varies in different groups in 40 pregnancies. Fetuses with ultrasound anomalies showed higher diagnosis rate (6/10, 60%) relative to other groups. The families having a history of abnormal pregnancy or having a proband deceased with undiagnosed were diagnosed in 3 of 12 cases (25%). Then we focused on analyzing WES performance in different phenotypes with ultrasound anomalies, including malformation of the central nervous system (CNS), cardiovascular system, skeletal system, genitourinary system and craniofacial malformation and multiple malformations(table 3). We could see that the 2 fetuses with skeletal system malformation were all got to be diagnosed and detection rate reached 100%.while the

cases having multiple malformations just got detection rate of 33%(1/3), and all 2 cases with genitourinary system abnormalities remained to be undiagnosed.

DISCUSSION

There are still great challenges for prenatal diagnosis of fetuses with suspected genetic disorders because of incomplete fetal phenotype information. Therefore, the prenatal diagnose rate is still lower than postnatal rate. A recent systematic literature review (2014 to 2017) revealed WES technology presented a broad range (6.2–80%) of diagnostic yield in fetuses with structural anomalies depending on the inclusion criteria [7]. Herein, we present the prenatal detection results in one center and showed that WES could detect 20% positive rate in the unselected fetuses which is similar to the study published in lancet this year [2,3]. While the most important difference is inclusion criteria. Not only fetuses with structural abnormalities were included in our study, but the other three groups were all covered including fetuses with UPD found by CMA, normal prenatal diagnosis results of single gene disorders, history of adverse pregnancy or proband deceased with undiagnosed, parents with undetermined etiology disease. Thus, the range of families underwent WES test in prenatal diagnose became more extensive and comprehensive here. However, it's worth mentioning that families with the patient of diagnosed single gene disorders are more likely to choose WES test even their fetuses have been diagnosed not likely to be a patient as the proband. And the WES results intended to be negative. ES achieved molecular diagnostic rates of 100% in all 2 fetuses with skeletal system anomaly which is consistent to the reported studies. In addition, there were several cases in table 1 which should be discussed in detail. In case 1, 22q11.2 deletion syndrome was identified by WES-CNV analysis and intended to be paternally inherited, which was confirmed by CNV-seq eventually. Similar results were also found in case 9. WES-CNV analysis results suggested the fetus inherited DMD gene of exon 46-50 deletion from their father. Hence, ES is useful for the detection of copy number variation in exon level, although more evidence is still needed. In case 11, genodermatoses related genes were sequenced using designated panels (panel sequencing) for proband three years ago who was diagnosed with xeroderma pigmentosum (XP) in our center, but a diagnosed result was not achieved at the time. Now Trio WES was conducted using fetus–mother–father samples and we found that parents of the proband were heterozygotes of *ERCC5* gene variants and carried one mutant allele separately. *ERCC5* gene was a pathogenetic gene of XP disease. Then we reanalyzed earlier genetic sequencing data of proband and found proband indeed harbored these two heterozygotes of *ERCC5* gene variants. It was suggested that the proband's XP disease resulted from the compound heterozygous variants in*ERCC5* gene. Actually, Liu et.al reanalyzed data from two patient series that had undergone diagnostic proband-only whole exome sequencing and found reanalysis of clinical whole exome sequencing data would significantly improve diagnostic yields[8].At last, we analyzed the genetic variants information of the diagnosed cases. All 8 diagnosed cases had inherited the relevant variants (5 variants were autosomal recessively inherited and 3 were autosomal dominant). Nevertheless, more population numbers received WES diagnose are needed to obtain a confident result.

In conclusion, the present study reveals that WES has the potential to improve the clinical management of pregnancies and provide risk of recurrence in future pregnancies. Further research is required to determine in which subgroups WES may have the greatest clinical utility before WES could become part of routine prenatal testing.

Disclosure of interests

The authors declare no conflicts of interest.

Contribution to authorship

Zhu conceived and planned the experiments. Liu and Zhao carried out the experiments. Shi contributed to sample preparation. Bai contributed to the interpretation of the results. Zhu wrote the paper. Kong reviewed and edited the manuscript. All authors read and approved the manuscript.

Details of ethics approval

Research ethics board approval was obtained from the Human Ethics Committee of the first affiliated hospital of Zhengzhou university (KS-2018-KY-36).

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Case	**Gestational weeks**	**clinical diagnosis**	**Detection method**	**Test results**	**Genetic variants**	**Pregnancy Outcome**
1	20	pulmonary atresia with ventricular septal defect	trio ES	22q11.2 microdeletion syndrome, paternally	22q11.21 deletion	Termination
2	30	history of two adverse pregnancies, bilateral pleural effusion, ascites, subcutaneous edema thickening	trio ES + CNV-seq	Small nuclear myopathy patient with ophthalmoplegia caused by two complex heterozygous variants of *RYR1* gene	*RYR1* c.844C>T(p.R282W) c.9472+1G>A	Termination

3	16	Proband having eye disease with retinal degeneration, wrinkled skin, poor vision	proband + trio-ES + CNV-seq	No P/LP variants were found with fetal while probands were diagnosed as exudative vitreoretinopathy patients with two complex heterozygous variants of LRP5 gene	*LRP5* c.3901G>A(p.A1301T) c.3361A>G(p.N1121D)	To be continued
4	28	The first fetus with talipes Varus, hydronephrosis, hydrocephalus, the second child with talipes Varus, hydrocephalus	trio ES + CNV-seq	Likely pathogenic variants of *POLG* gene were found	*POLG* c.868C>T(p.R290C) c.2890C>T(p.R964C)	Termination
5	18	abortion three times with encephalocele	trio ES	Likely pathogenic variants of *C5orf42* gene were found	*C5orf42* c.8673_-8676del(p.K2891fs) c.374delA(p.N125fs)	Termination
6	18	Last fetus with less amniotic fluid, larger renal volume, suspected infantile polycystic kidney disease	trio ES + CNV-seq	Fetus was diagnosed as PKHD1 gene c.5935G> A (p.G1979R) heterozygous mutation carrier, maternal inherited; proband was suggested to be a patient with PKHD1 gene compound heterozygous variants	*PKHD1* c.5935G>A(p.G1979R) c.1748_-1749delGTinsAA(p.C583X)	To be continued

7	24	mandibular retrog-nathia, more amniotic fluid, the last fetus having a small jaw and cleft palate	trio ES + CNV-seq	Fetus was suspected to be a patient of *COL2A1* gene c.2491G> T (p.G831X) heterozy-gous variants, paternal inherited.	*COL2A1* c.2491G>T(p.G831X)	Termination
8	32	P1 fist with both hands, both feet close, died a day later after birth. P2 abnormal flexion with one hand, to close one leg. P3 more amniotic fluid, sustained handgrip hands, abnormal toes	trio ES + CNV-seq	Suspected causative mutation of FBXO11 gene c.1330G> A (p.V444I), heterozygous	*FBXO11* c.1330G>A(p.V444I)	To be continued
9	19	Husband is a DMD patient with DMD gene exon 46-50 deletion	trio ES + CNV-seq	twins are both carriers of heterozy-gous deletion of exon 46-50 in *DMD* gene	Xp21.1 deletion	To be continued
10	12	MMA child birth history	trio ES + CNV-seq	Carrier of MMACHC gene C.217C>T mutation, maternal inherited	*MMACHC* c.217C>T(p.R73X) c.656_658del (p.Lys220del)	To be continued

11	18	Proband having microcephaly and mental retardation was suspected to xeroderma pigmentosum disease patient	trio ES + CNV-seq	Carrier of ERCC5 gene c380G>A mutation, maternal inherited; proband was confirmed to be a xeroderma pigmentosum patient caused by *ERCC5* gene compound heterozygous mutation	*ERCC5* c.380G>A(p.R127K) c.1172dupT(p.I391fs)	To be continued
12	27	child birth history with POMT1 variants caused disease, pregnant woman herself was a patient	trio ES + CNV-seq	Carrier of POMT1 gene c.2210-2221del mutation inherited from mother	*POMT1* c.2164G>A(p.G722R) c.2210_-2221del(p.K737_-D741delinsN)	To be continued

Table 1 summary of 12 diagnosed cases including four variants carriers of AR inheritance

Table 2 clinical diagnosis rate varies in different groups in 40 pregnancies

clinical diagnosis	The number of cases	Positive cases	The positive rate
Fetuses with UPD found by CMA	1	0	0
Fetuses with Abnormal ultrasound	10	6	60%
Normal Prenatal diagnosis results of single gene disorders	14	0	0
History of adverse pregnancy or proband deceased with undiagnosed	12	3	25%
Parents with undetermined etiology disease	3	0	0

Table 3 Diagnostic rates in fetuses with different ultrasound abnormalities

Systems affected	the number of cases	Positive cases	The positive rate
Skeletal system	2	2	100%

Multisystem disorder	3	1	33%
CNS	1	1	100%
Cardiovascular System	1	1	100%
Facial abnormalities	1	1	100%
Genitourinary	2	0	0%