


Usefulness of copy number variant detection following monogenic disease exclusion in prenatal diagnosis

Panlai Shi, Yanjie Xia, Qianqian Li and Xiangdong Kong 

Genetic and Prenatal Diagnosis Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Abstract

Aim: Families with an adverse history of monogenic disease focus on single-gene diagnosis instead of low-depth whole-genome sequence, during subsequent pregnancies. The aim of this study was to assess the potential usefulness of low-depth whole-genome sequencing (copy number variant sequencing [CNV-seq]) detection following monogenic disease exclusion in prenatal diagnosis.

Methods: A total of 285 families with a history of monogenic disease (of 41 different types; eliminated during the current pregnancy) were recruited and retrospectively analyzed. Low-depth whole-genome sequencing (CNV-Seq, Next-Seq CN500 platform) was performed for all fetuses.

Results: The CNV detection results of the 285 samples were as follows: one case of 18-trisomy chimera (0.35%), one case of pathogenic 3q29 microdeletion syndrome CNV (0.35%), four cases of variant of uncertain significance (VUS) CNVs (1.40%), and four cases of Duchenne muscular dystrophy (DMD) carriers (1.40%); and the remaining samples were normal (96.15%). Of note, 2/285 (0.70%) samples still exhibited pathogenic abnormalities. All positive samples were followed up where the two cases of pathogenic abnormalities elected the pregnancy termination, while the four VUS cases and four DMD-carrier cases were born healthy.

Conclusion: In cases where prenatal fetal monogenic disease has been ruled out, CNV detection is still beneficial and should be performed to prevent missed pathogenic CNVs. However, the costs need to be balanced against benefits, and the research will need to assess other types of testing.

Key words: copy number variant, copy number variant sequencing, monogenic disease, prenatal diagnosis.

Introduction

With the development of high-throughput technology, an increasing number of monogenic disease mutation sites have been identified, and the demand for prenatal diagnosis is increasing. Prenatal diagnosis of monogenic diseases has become routine in China. Almost all monogenic diseases are rare diseases. Guidelines of diagnosis and treatment of rare diseases (2019) were issued by National Health Commission of People's Republic of China. The guidelines include

overview, etiology and epidemiology, clinical manifestations, auxiliary examinations, diagnosis, differential diagnosis, treatment and diagnosis flowchart of 121 monogenic diseases.

There are usually two types of prenatal diagnosis results: (i) the fetus is affected, in which case the family usually chooses to terminate the pregnancy, and (ii) the fetus is a carrier or healthy, in which case the family will usually continue with the pregnancy. In general, genetic counseling for carriers of monogenic disease focuses on fertility (natural or assisted

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Correspondence: Dr Xiangdong Kong, Director of Genetic and Prenatal Diagnosis Center, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China. Email: kongxd@263.net

Panlai Shi, Yanjie Xia, and Qianqian Li contributed equally to this work.

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reproduction), genetic inheritance, prenatal diagnosis, and counseling for their offspring, whereas, genetic counseling for pregnant individuals with a carrier or healthy fetus would follow conventional process including prenatal screening or prenatal diagnosis.

In addition to testing for single-gene disease, prenatal diagnostic techniques include chromosome karyotype analysis, chromosome microarray analysis (CMA), low-depth whole-genome sequencing (CNV-Seq), whole exome sequencing (WES) and whole genome sequencing (WGS). However, whether fetuses with a 'normal' prenatal diagnosis require further chromosomal analysis and how best to perform these tests remains a concern for both doctors and families.

Genome copy number variants (CNVs) are copies of DNA sequences that are usually >1 kb in size, which can result in microdeletions, microduplications and complex rearrangements of the genome.¹ CNVs have been found to play a vital role in an increasing number of human diseases such as autism, schizophrenia and epilepsy.² Pathogenic CNVs have been shown to cause >300 types of chromosomal microdeletion and microduplication syndromes,² with an overall incidence of approximately 1/600,^{3,4} accounting for 50% of birth defects caused by chromosomal abnormalities.⁵ Low-coverage massively parallel CNV sequencing (CNV-seq) technology is based on whole-genome next-generation sequencing technology⁶ and

has been established as a suitable first-tier diagnostic test for detecting clinically significant fetal chromosome anomalies.⁷ However, it remains unclear whether pregnant women with a previous monogenic-disease birth or family history, which have been ruled out in the current pregnancy, requires CNV testing.

As CNV-Seq has optimum resolution of CNV and is affordable for patients. Hence, the aim of this study was to assess the potential usefulness of CNV detection following monogenic disease exclusion in prenatal diagnosis by CNV-Seq.

Methods

Subjects

A total of 774 pregnant women with a history of monogenic disease birth, treated at the Department of Genetics and Prenatal Diagnosis of the First Affiliated Hospital of Zhengzhou University from January 2017 to December 2019, were recruited. The flowchart of prenatal diagnosis process in 771 families with monogenic diseases was showed in Figure 1. And, 285 of 593 'normal' group added the CNV testing. The average age of the women was 31.4 years (range: 19–46). The average gestational age of the women was 14 weeks (range: 11–29). The subjects were divided

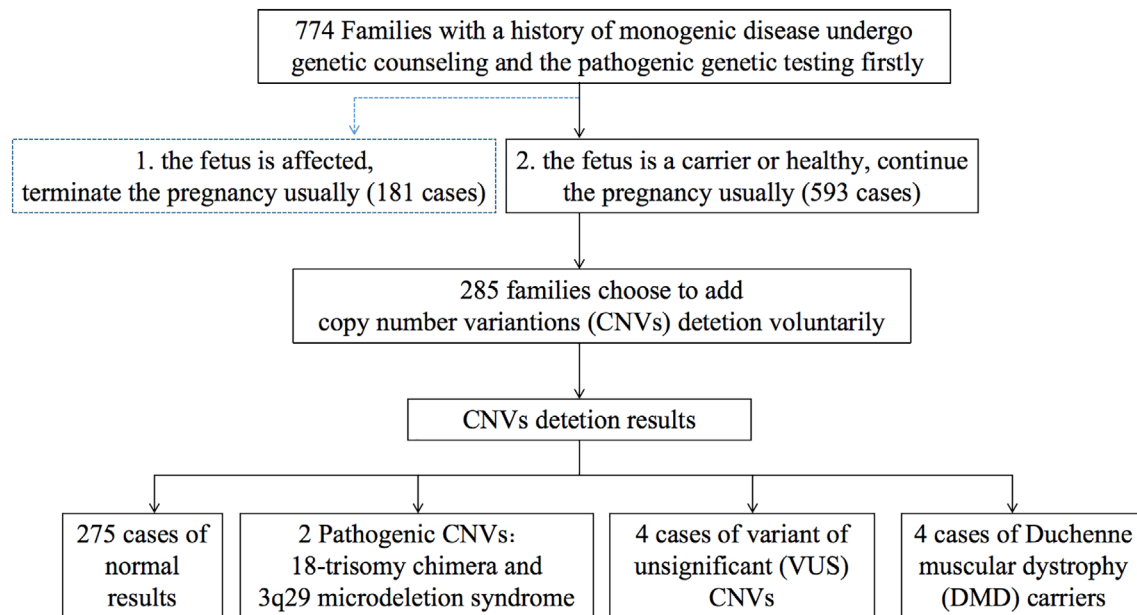


Figure 1 The flowchart of prenatal diagnosis process in 285 families with monogenic diseases.

Table 1 Abnormal CNVs results in 285 fetuses with monogenic diseases excluded

Monogenic disease	CNV-seq results	Category	Pregnancy outcomes	Age at follow-up
DMD	47,XN,+18(mos)	2 pathogenic CNVs	Termination of pregnancy	NA
SMA	3q29(195740000-197400000)x1, 1.66 Mb			NA
DMD	22q11.21(18600000-20740000)x3, 2.14 Mb	4 VUS CNVs	Born healthy	1 year and 7 months
PKU	5q13.3q14.1(76600000-77820000)x3, 1.22 Mb			7 months
Deafness	22q11.21(18500000-19040000)x3, 0.54 Mb	4 DMD carriers	Born healthy	10 months
IRD	18p11.31p11.23(6580000-7360000)x3, 0.78 Mb			5 months
DMD	46,XX,Xp21.1(31980000-32180000)x1, 0.20 Mb			1 year and 9 months
	46,XX,Xp21.1(31840000-32080000)x1, 0.24 Mb			2 years
	46,XX,Xp21.1(32630000-33010000)x1, 0.38 Mb			1 year and 7 months
	46,XX,Xp21.1(31800000-32040000)x1, 0.24 Mb			

CNVs, copy number variant; DMD, Duchenne muscular dystrophy; IRD, immunodeficiency-related disease; PKU, phenylketonuria; SMA, spinal muscular atrophy; VUS, variants of uncertain significance.

according to monogenic disease category as Table S1. All CNV detection results are summarized in Table 1. The above-mentioned diseases were ruled out in the fetuses by Sanger sequencing or multiplex ligation-dependent probe amplification (MLPA) and the pregnant women voluntarily requested additional fetal CNV testing. All subjects provided informed consent for prenatal genetic investigation and were approved by the Zhengzhou University Ethics Committee.

Genetics counseling

Genetic counseling for the candidate women before prenatal diagnosis using CNV-seq mainly contains the scopes and limitations of CNVs testing, especially for the possible detection of pathogenic CNV of incomplete penetrance, variant of uncertain significance (VUS) CNV, and further required parent-origin detection.

Samples

Chorionic villus sampling or amniocentesis was performed as previously described.⁸⁻¹⁰ Maternal peripheral blood samples were collected to eliminate maternal contamination. Genomic DNA was extracted from peripheral blood, chorionic villus, and amniocytes collected by centrifugation and washed with phosphate buffered saline using the DNeasy Blood and Tissue Kit (Qiagen). Quantitative fluorescent PCR (QF-PCR) was used as the quality control to detect all DNA contamination. Short tandem repeat (STR) markers were used for chromosome 21 (D21S1435, D21S1411, D21S11), chromosome 18 (D18S1002, D18S391, D18S535, D18S386), chromosome 13 (DXS981, DXS6809, DXS22), and sex chromosomes X and Y according to the operating procedures.⁷

CNV-Seq

CNV-seq was performed as previously described.⁷ The identified CNVs (BLAST with hg19) were queried against public databases, including but not limited to DGV (<http://dgv.tcag.ca/dgv/app/home>), gnomAD (<https://gnomad.broadinstitute.org/>), DECIPHER (<https://decipher.sanger.ac.uk/>), OMIM (<https://www.omim.org/>), UCSC (<https://genome.ucsc.edu/>) and ClinGen (<https://dosage.clinicalgenome.org/>), and pathogenicity was assessed according to the latest guidelines outlined by the American College of Medical Genetics (ACMG).¹¹ CNVs were classified into five levels: benign, likely benign, VUS, likely pathogenic, and pathogenic.

Results

One case of 18-trisomy chimera and four *DMD* carriers were detected among 123 prenatal cases excluded for *DMD* disease

DMD is an X-linked recessive inherited neuromuscular disease caused by mutations in the disease-causing gene *DMD*. *DMD* mutation types include deletion (55–65%), duplication (5–10%), point mutation (25%) and other types (approximately 8%).¹² Of the 123 cases excluded for *DMD* disease, CNV-Seq detected one case of 18-trisomy chimera and four *DMD* carriers.

In the 18-trisomy chimera case, the 33-year-old pregnant woman already had a son with *DMD* and was particularly anxious as to known whether the fetus had *DMD* disease. Although the fetus was excluded for *DMD* using MLPA, suspected 18-trisomy was detected using QF-PCR and 18-trisomy chimera was confirmed in CNV-seq (Fig. 2a,b). The chimera ratio was 70%, as shown in Figure 2. The family chose to terminate the pregnancy following adequate genetic counseling.

In the 4/123 cases diagnosed as carriers of *DMD* heterozygosity by MLPA, a heterozygous deletion at Xp21.1 was also revealed using CNV-Seq. The deleted fragment contains the *DMD* gene (Table 1 and Fig. 3 [CNV results of one case]).

One case of 3q29 microdeletion syndrome was found among 31 cases excluded for SMA disease

A 34-year-old pregnant woman with a normal ultrasound at 12 weeks of gestation underwent chorionic villus sampling for SMA testing. Although the results were SMA-negative, a 1.66 Mb deletion in 3q29 (195 740 000 – 197 400 000) was detected by CNV-seq (Fig. 4). The deletion region contained 23 protein-coding genes, including 19 Online Mendelian Inheritance in Man (OMIM) genes, and spanned 3q29 recurrent microdeletion region (including the Discs large homolog 1 gene, *DLG1*; chr3: 195756054 – 197 344 662), with sufficient haploinsufficiency dosage pathogenicity (ISCA-37443). The haploinsufficiency phenotype was 3q29 deletion syndrome. The clinical manifestations of 3q29 microdeletion syndrome vary greatly, from mild to moderate developmental delay, autism disorder, intellectual disability, language developmental delay and microcephaly.^{13,14} Therefore, the family chose to terminate the pregnancy after adequate genetic counseling.

Four VUS CNVs were detected among a total of 285 cases

VUS CNVs represent a broad category with no identified evidence proving pathogenicity; these CNVs require further investigation and testing of parental origin. In our study, four cases of VUS CNVs were detected among 285 cases. One case of 5q13.3q14.1 duplication, one that of 18p11.31p11.23 duplication, and two cases of 22q11.21 duplication; all inherited from the parents with normal phenotype. Hence, the families chose to continue the pregnancies. Four cases of VUS CNVs and four *DMD*-carrier cases were born healthy and were followed up for 7 months to 2 years.

Discussion

Monogenic disease is a type of genetic disease caused by a single gene mutation; it includes >10 000 types, most of which are rare diseases, with a total incidence of approximately 2–3%.^{15,16} Currently clinical testing of monogenic disease genes is carried out using Sanger sequencing, MLPA, gene-panels, next-generation gene sequencing, whole-exome sequencing, whole-genome sequencing and third-generation sequencing technology. Sanger sequencing, MLPA and gene-panels are the most commonly used methods for monogenic diseases with identified causal genes, as they are economical and fast. In addition, a series of diagnostic methods and strategies have been introduced for monogenic diseases.¹⁷ However, to date, there is no prenatal diagnosis strategy once monogenic diseases are excluded.

As CNV-seq has been demonstrated to be a suitable first-tier diagnostic test for detecting clinically significant fetal chromosome anomalies,⁷ we performed a retrospective analysis including 285 families in which prenatal monogenic diseases had been excluded and that had undergone additional voluntary CNV testing. We found that a 0.70% risk of abnormal pathogenicity (one case of 18-trisomy chimera and one case of 3q29 microdeletion syndrome) remained, and four cases of VUS CNVs (0.14%) and four *DMD*-carrier cases (0.14%) were observed.

At present, few cases of 3q29 microdeletion have been identified in fetuses in China and abroad. Zhang *et al.* reported a prenatal diagnosis of an intra-uterine growth retardation fetus with 3q29 deletion syndrome, which constitutes a new variant,¹⁸ and

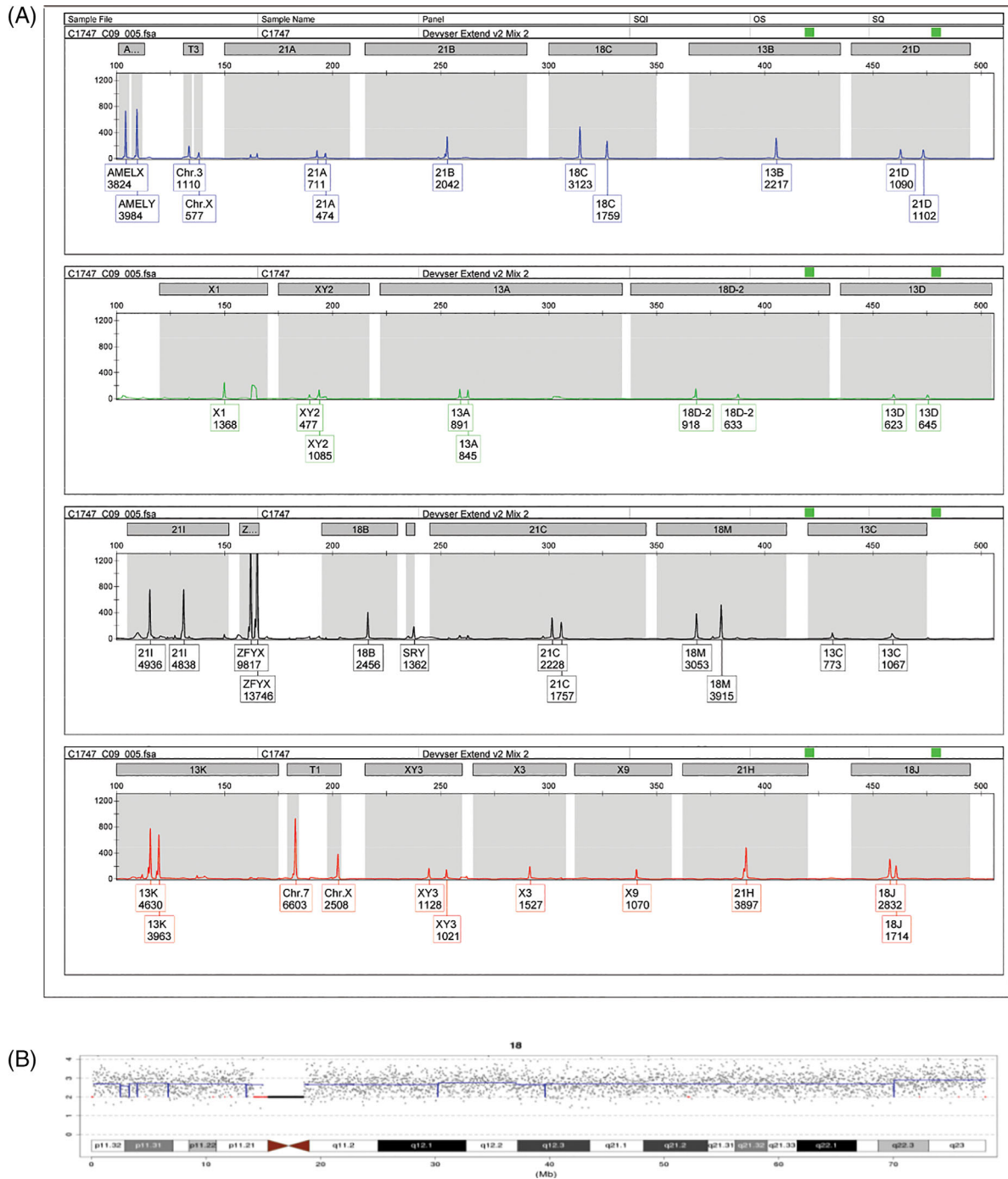


Figure 2 The results of 18-trisomy chimera detected by QF-PCR (a) and CNV-seq (b). The chimera ratio is about 70%.

Long *et al.* reported two cases of 3q29 deletion syndrome fetuses. A previous study found that most 3q29 deletions are *de novo*, and few cases are inherited from parents with phenotypes.¹⁹ The main clinical symptoms are ventricular septal defect and

cleft lips and palate. However, no abnormalities were observed using ultrasound in our study. Coe *et al.* observed a 3q29 deletion in 11/29 085 cases of stunted children and 0/19 584 of the control group.^{20,21}

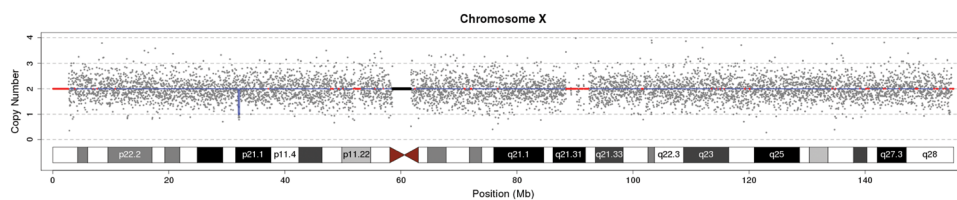


Figure 3 A case of CNVs detection results of a DMD carrier detected by CNV-seq.

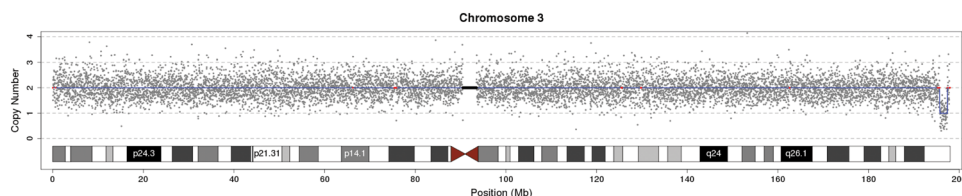


Figure 4 3q29 microdeletion from 195 740 000 to 197 400 000 detected by CNV-seq.

In the four cases of female carriers of *DMD* heterozygosity, CNV detection showed a minimum of 200 kb and a maximum of 380 kb heterozygosity at Xp21.1, which was consistent with the original *DMD* MLPA test results. This indicates that CNV-Seq can verify heterozygous *DMD* carriers, which is consistent with the reported *DMD* detection results using single nucleotide polymorphism (SNP) arrays or chromosome microarray.^{22,23}

In addition, all positive samples were followed up: the two pathogenic cases underwent induced labor, while the four VUS cases and four *DMD*-carrier cases were born healthy.

As a gray area, VUS CNV brought a great challenge and difficulty for clinical genetic consulting. In addition, it may take the pregnant women and her family anxious. Currently, it is necessary to investigate the prenatal to further interpret the CNV in fetus. According to the research, 87.46% VUS were inherited from parents and there is no significant difference in abnormal pregnancy outcomes in the inherited, *de novo*, and refusal groups.²⁴ And, in our study, all four VUS CNV were found to be inherited from parents with normal phenotype and were born healthy. In addition, pregnancy is a dynamic and long-term process, which needs comprehensive observation combined with ultrasound and other indicators. Therefore, the thorough and detailed informed consent is necessary. It is an urgent requirement to establish a database of VUS that includes features such as fetus ultrasound data, parental origin sources, pregnant outcome and postnatal development. This will provide a detailed guidance for interpreting VUS CNV more accurately.

Our results demonstrate that adding CNV detection can help prevent birth defects, as well as missed detection of pathogenic CNV in families in which the fetal single-gene disease has been ruled out. Thus, it was recommended to add the CNV detection in prenatal diagnosis guidelines of single disease. However, this diagnostic benefit needs to be balanced within a financial cost analysis as 309 families (51.9%) refused the test mainly due to the financial constraints. In 2019, per capita national income of the China was \$10 410. The CNV testing costs about \$365. It's relatively expensive compared to the mean income. In addition, the limitation of this study lies in the number of family cases. In the future, the number of studies will be expanded and statistics will be analyzed again.

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Disclosure

None declared.

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Supporting information

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Table S1. The category of monogenic diseases indicating prenatal diagnosis.