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# BMJ Open

## Protocol for a clinical trial of comprehensive non-invasive prenatal testing for pregnancies with elevated risks of genetic disorders

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# Protocol for a clinical trial of comprehensive non-invasive prenatal testing for pregnancies with elevated risks of genetic disorders

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**ABSTRACT**

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Introduction

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Chromosomal abnormalities and monogenic disorders account for ~15-25% of recognizable birth defects. With very limited treatment options, preconception or prenatal screening was developed to reduce the incidence of such disorders. Currently, non-invasive prenatal screening (NIPS) for common aneuploidies is implemented worldwide with proven clinical utility. However, the clinical validity for screening of frequent chromosome segmental copy number variations (CNVs) and monogenic disorders still awaits to be investigated through a prospective and evidence-based approach. This prospective clinical trial aims to assess the clinical accuracy of a novel NIPS test for concurrent screening of aneuploidies, CNVs and monogenic diseases.

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Methods and analysis

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This study is a multicenter trial and participants will be recruited from three tertiary hospitals in China. Pregnant women with abnormal prenatal screening results indicated for invasive prenatal diagnosis or those who decided to terminate their pregnancies due to abnormal ultrasound findings will be evaluated and enrolled with informed consent. Genetic counseling will be provided for participants who receive confirmed positive testing results. Plasma specimens will be collected from ~1,000 participating pregnancies which are divided according to their respective indications. Cell-free DNA will be analyzed by an analytically validated NIPS test to generate comprehensive screening results. The diagnostic results from prenatal invasive specimens or postnatal

specimens will be collected to examine the accuracy of the screening results. The pregnancy outcomes will be evaluated to assess the clinical validity, and the clinical utility will be evaluated based on the pregnancies impacted by the screening results. The benefits and limitations of this test will also be explored especially for any potential issues in genetic counseling.

## Ethics and dissemination

This study was approved by the Obstetrics and Gynecology Hospital of Fudan University (2020-178). Results of this study will be submitted for publication in a peer-reviewed journal.

## Trial registration number

ChiCTR2100045739.

## Strengths and limitations of this study

- The first study on an integrated screening of both chromosomal abnormalities and monogenic diseases.
- This is a prospective study involving multi-center and stratified samples.
- The criteria need to be studied on selection of indications for high-risk pregnancies
- The potential loss of postnatal follow-up will interfere data completeness.

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66 INTRODUCTION

67 Genetic etiology of birth defects

68 Birth defects are congenital conditions causing structural or functional anomalies at  
69 birth, which greatly contribute to infant mortality and disability[1]. Approximately 3–5% of  
70 newborns are affected by a birth defect worldwide[2]. Although the causes of most  
71 cases are unknown, about 15-25% of birth defects are due to genetic diseases such as  
72 chromosomal abnormalities and monogenic disorders[3].

74 The screening of severe genetic diseases

75 Great efforts have been made to prevent birth defects with underlying genetic etiology.  
76 Carrier screening for recessive disorders such as Tay-Sachs disease was proved to be  
77 highly effective for the reduction of disease incidence[4]. The first-trimester combined  
78 screening for fetal aneuploidies by prenatal ultrasound and maternal serum biochemical  
79 testing is able to detect over 85% common trisomies at a false positive rate of ~5%  
80 which can lead to parental anxiety and sometimes unnecessary invasive diagnostic  
81 procedures with the risk of pregnancy loss[5 6]. Since the discovery of circulating fetal  
82 cell-free DNA (cfDNA) in the maternal plasma during pregnancy, its biological  
83 characteristics and clinical implication have been extensively explored[7 8]. Non-  
84 invasive evaluation for fetus' gender and risks for monogenic disorders, aneuploidies,  
85 and chromosome segmental CNVs were developed using different molecular or  
86 genomic techniques[9-11]. Importantly, the emergence of next-generation sequencing  
87 (NGS) technology enabled a practical screening method for Down syndrome [12 13]. In

the past decade, NGS based non-invasive prenatal screening (NIPS) for trisomy 21, 18, and 13 has become a new standard for prenatal care with proven clinical utility[14]. Recently, NIPS was used to detect rare autosome trisomies, sex chromosome aneuploidies, and microdeletion or microduplication syndromes which showed potential clinical utility for the prenatal management of these disorders[15 16]. However, monogenic disorders which represent another major cause of birth defects are beyond the scope of the current screening of chromosomal abnormalities, and the clinical validity and utility for the screening of the monogenic diseases are yet to be demonstrated.

The development of NIPS for monogenic disorders

Previous studies have shown that the analysis of fetal cfDNA was useful to determine the inheritance of parental alleles associated with autosomal or sex-linked recessive monogenic diseases [17 18]. Additionally, non-invasive prenatal testing was also accurate for the diagnosis or screening of the *de novo* or paternally inherited variants causing dominant diseases such as achondroplasia and Noonan spectrum disorders[19 20]. These studies demonstrated the potential clinical utility of monogenic NIPS (NIPS-M) in pregnancies at moderate risks (e.g., pregnancies with advanced paternal age or ultrasound soft markers). These tests could also be used for the screening of diseases which can only be discovered at late gestational ages (e.g., skeletal disorders).

Although the analytical validity of NIPS-M has been well documented, such tests will not be widely accepted without further evidence-based study to prove its clinical validity and utility[21]. Firstly, the clinical validity of NIPS-M has not been supported by large

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111 prospective studies. The follow-up studies of the pregnancies tested positive or negative  
112 should be evaluated by clinical examination or golden standard diagnostic tests.  
113 Secondly, the clinical utility of NIPS-M has not been thoroughly exploited. Although  
114 isolated cases have demonstrated the benefits of NIPS-M leading to early diagnosis  
115 and better prenatal/postnatal management, the benefits of such tests for appropriate  
116 indications are yet to be proven by larger studies[20 22 23]. Thirdly, the limitations of  
117 NIPS-M need to be evaluated. Accurate genetic counselling is critical to the success of  
118 a prenatal screening test which should provide information regarding disease  
119 characteristics, natural history, penetrance, expressivity, genotype-phenotype  
120 correlation, and etc. The benefits and risks of NIPS-M need to be carefully evaluated  
121 when patients are counseled based on current understanding of the disease[24].  
122 This trial is aimed to address above important issues regarding the clinical validity and  
123 utility of an innovative NIPS for the concurrent screening of common aneuploidies,  
124 CNVs and monogenic disorders.  
125  
126 Aims  
127 1. To assess the clinical validity of a novel NIPS test for concurrent screening of seven  
128 common aneuploidies, nine microdeletion and microduplication syndromes (MMS) and  
129 155 monogenic disorders (75 genes).  
130 2. To evaluate the clinical utility of this comprehensive NIPS by studying whether the  
131 clinical management of the screened pregnancies are affected.

3. To investigate genetic counseling and other pregnancy management issues due to the limitation of this comprehensive NIPS.

## METHODS AND ANALYSIS

### Study design

This prospective cohort study aims to evaluate how comprehensive screening of both chromosomal and monogenic disorders will reveal pregnancies at risk and affect the management of high-risk pregnancies. It is a multicenter trial focusing on pregnant women with indications for prenatal diagnosis, including fetal ultrasound markers, high risk results by maternal serum screening or routine NIPS. Pregnancies with elective abortion due to fetal structural abnormality will also be recruited. After the NIPS test, cases will be followed up to compare the screening results with the prenatal or postnatal diagnostic test results including sequencing, chromosomal CNV testing and/or karyotyping. Clinical follow-up will be pursued regarding the pregnancy outcome up to 6 weeks after birth. The NIPS performance metrics, and the clinical benefits for pregnancies impacted by the comprehensive non-invasive screening results will be evaluated (Figure 1).

### Consent and eligibility

Participants will be recruited from three tertiary hospitals in China including the Obstetrics and Gynecology Hospital of Fudan University (Shanghai), the Hunan

Provincial Maternal and Child Health Care Hospital (Changsha), and the Women's Hospital of Zhejiang University (Hangzhou). The detailed descriptions of inclusion and exclusion criteria are shown in Table 1. Pre-test genetic counseling will be provided by the healthcare provider to all participants prior to obtaining the written informed consent to complete registration. The purpose and process of this study, as well as benefits, risks, data privacy and rights to withdraw will be discussed during the counseling session.

## Patient and public involvement

Patients or the public were not involved in the design, conduct, or dissemination plans of this research.

## Sample size

This study aims to recruit a total of 1,000 pregnancies undergoing invasive diagnostic procedures or elected abortions due to abnormal prenatal screening findings suggestive of elevated risks for severe genetic disorders. All eligible samples will be stratified into different group depending on the indication of high risk (Table 1), and the number of samples in each group will be approximately allocated as the following: Group 1, fetal structural anomalies detected by ultrasound (60%); Group 2, high risk by routine NIPS (20%); Group 3, high risk by maternal serum biochemical testing (10%); Group 4, suspected genetic causes with other indications (10%). For group 1, further stratification will be achieved based on the gestational age (Table 1), including 12-16 weeks (60%),

175 17-21 weeks (20%), and 22 weeks and above (20%). The number of samples from  
176 each hospital will be evenly collected given equal participant availability.

## 178 Screening and reporting

179 The comprehensive non-invasive prenatal screening test used in this study is based on  
180 liquid-phase target enrichment followed by high read-depth NGS. This test can  
181 concurrently detect seven common chromosomal aneuploidies, nine MMS, and 155  
182 monogenic disorders (Table 2). For testing procedure, 10 ml of peripheral blood is  
183 collected from each participant and the plasma is separated through a two-step  
184 centrifugation process. Cell-free DNA extraction and NGS library construction are  
185 performed according to the manufacturer protocol (TIANGEN and Nanodigbio, China).  
186 Custom-designed hybridization probes are synthesized and used for target enrichment  
187 (Integrated DNA Technologies, USA). The final DNA library is sequenced at 2x100  
188 paired-end mode on MGISEQ-2000 sequencer (MGI, China).

189 For both chromosomal and monogenic findings, the pathogenicity of copy number or  
190 monogenic variant is evaluated according to the ACMG guidelines[25 26]. Only  
191 pathogenic and likely pathogenic variants are deemed positive and reportable to  
192 primary care providers and the results will be reported to the patients after diagnostic  
193 confirmation. For cases with chromosomal abnormalities identified in the NIPS test,  
194 karyotyping, chromosomal microarray analysis (CMA), and whole genome sequencing  
195 for copy number variation analysis (CNV-seq) are used as diagnostic tests. For cases

196 with screening positive monogenic variants, Sanger sequencing is used as the  
197 confirmatory test.  
198 Post-test genetic counseling will be provided to participants by experienced clinical  
199 geneticists regarding the interpretation of the screening and/or diagnostic results, the  
200 implications of these positive findings, and potential management options.

201

## 202 Pregnancy outcomes follow-up

203 All screening and diagnostic testing results, clinical examination results and images,  
204 and other relevant information are kept in medical records and used for statistical  
205 analysis. Pregnancies are followed up with outcomes such as elective abortion,  
206 miscarriage, stillbirth, or live born. Results of prenatal invasive diagnostic testing and  
207 testing of products of conception are collected, and those cases without confirmatory  
208 genetic testing are excluded from analysis. In addition to their birth records, newborns  
209 will undergo clinical examination or diagnostic testing if needed until 6 weeks after birth,  
210 and newborns with normal clinical examination results are considered to be negative if  
211 no genetic testing is performed. Results of fetal and newborn genetic testing are  
212 reviewed and classified according to the type of abnormality by at least two clinical  
213 geneticists.

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## 215 Statistical analysis

The diagnostic testing and pregnancy outcome results of all pregnancies will be used to define true positive and true negative, and compared with the results generated by comprehensive non-invasive screening. The outcome is the area under the receiver-operating-characteristic (ROC) curve (AUC) for detection of each type of abnormalities (aneuploidies, MMS, and monogenic disorders) by comprehensive non-invasive screening in high-risk pregnancies. The ROC curve is generated by computation of test sensitivity and specificity, and confidence intervals are computed using the Clopper–Pearson method. Assay performance metrics will also be demonstrated by false positive rate, false negative rate, positive predictive value (PPV), and negative predictive value (NPV), according to each category of abnormalities (Figure 2). Data will be analyzed with respect to different group of indications for high risk pregnancies, as well as pregnancies at different maternal age or gestational age. Clinical utility will be evaluated based on the pregnancies impacted by the comprehensive non-invasive screening results.

## Trial conduct

In this study, data are collected from test requisition forms filled by participants and healthcare providers, medical records kept in each hospital, and postnatal follow-up results. Site monitoring of source data is performed following the trial monitoring plan. All information collected during the course of the trial will be kept strictly confidential. Information will be held securely on paper and electronically at the Obstetrics and Gynecology Hospital of Fudan University. Data safety will be reviewed on a regular basis to identify any safety concerns or trends.

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240 ETHICS AND DISSEMINATION

241 This trial is sponsored and approved by the Obstetrics and Gynecology Hospital of

242 Fudan University (2020-178). All recruited participants provide written informed consent

243 prior to being enrolled into the trial at each hospital. A manuscript with results of this

244 study will be published in a peer-reviewed journal, and data will be presented at

245 academic conferences.

246

247 DISCUSSION

248 This is the first study to evaluate the clinical validity and utility of an integrated prenatal

249 screening for both chromosomal abnormalities and monogenic disorders. Although

250 NIPS for common aneuploidies has been accepted by professional societies and widely

251 implemented in general pregnant population, NIPS for monogenic disorders requires

252 evidence-based investigation to prove its benefits for the improvement of pregnancy-

253 related health management. To investigate the clinical validity of this new NIPS test,

254 high-risk pregnancies are selected based on the prenatal screening results suggestive

255 of genetic diseases and stratified into different groups. Additionally, important issues

256 around the clinical utility of this test will be carefully examined. For instances, the criteria

257 for the selection of diseases, the proper indications for this test, genetic counseling and

258 pregnancy management options will be evaluated to weigh the benefits and risks when

259 offering a comprehensive NIPS. Overall, we will generate evidence-based study results

from this prospective and multicenter trial to evaluate the clinical utility of the next-generation NIPS for different categories of genetic diseases.

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## Contributors

JZ and H-FH conceived the study. JZ, H-FH, YW, CX and XC participated in the design of the study and drafting of the manuscript. CX, SC, QL, HX, DZ and HW participate in recruitment of participants and assessment of clinical outcomes. XC and JZ will design the statistical analysis plan and oversee statistical analysis. All authors critically reviewed and approved the manuscript for submission.

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306 of sample processing and high-throughput sequencing was supported by Beijing  
307 BioBiggen Technology Co., Ltd..

308

309 Competing interests

310 XC, JZ are employees or shareholders of Beijing BioBiggen Technology Co., Ltd.. The  
311 other authors declare no conflict of interest.

312

313 Patient consent for publication

314 Not required

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316 Ethics approval

317 This trial had been reviewed and approved by the Obstetrics and Gynecology Hospital  
318 of Fudan University (2020-178)

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322 Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none"> <li>➤ Adult pregnant woman (age <math>\geq 20</math> years)</li> <li>➤ Gestational age <math>\geq 12^{+0}</math> weeks</li> <li>➤ Singleton pregnancy</li> <li>➤ High-risk pregnancy as indicated by the following:               <ol style="list-style-type: none"> <li>1. Fetal structural anomalies detected by ultrasound including but not limited to:                   <ol style="list-style-type: none"> <li>12-16 weeks:                       <ol style="list-style-type: none"> <li>a. Increased nuchal translucency or cystic hygroma</li> <li>b. Cardiac structural defects</li> <li>c. Absence or hypoplasia of nasal bone</li> </ol> </li> <li>17-21 weeks:                       <ol style="list-style-type: none"> <li>a. polycystic kidney</li> <li>b. intrauterine growth restriction</li> <li>c. malformation of the digestive tract</li> <li>d. ventriculomegaly</li> <li>e. polyhydramnios</li> <li>f. oligohydramnios</li> <li>g. echogenic bowel</li> <li>h. pyelic separation</li> </ol> </li> <li>22 weeks and above:                       <ol style="list-style-type: none"> <li>Skeletal abnormalities detected in the following:                           <ol style="list-style-type: none"> <li>a. length, shape or mineralization of long bones</li> <li>b. number of fingers and toes</li> <li>c. shape of palms and soles</li> <li>d. circumference of head, abdomen or chest</li> <li>e. mineralization and shape of skull and spine</li> <li>f. size and shape of scapula, clavicle, forehead, nasal bone or mandible</li> <li>g. posture of limbs</li> </ol> </li> </ol> </li> </ol> </li> <li>2. High risk by routine NIPS</li> <li>3. High risk by maternal serum biochemical testing</li> <li>4. Suspected genetic causes such as recurrent miscarriage</li> </ol> </li> <li>➤ To perform invasive diagnostic procedure during pregnancy, and will take at least one molecular test on specimens such as amniotic cells, cord blood, product of conception, or newborn's peripheral blood</li> </ul>	<ul style="list-style-type: none"> <li>➤ Age <math>&lt; 20</math> years</li> <li>➤ Gestational age <math>&lt; 12^{+0}</math> weeks</li> <li>➤ Chromosomal abnormality in either of the couple</li> <li>➤ Received allogeneic blood transfusion, organ transplantation, or cell therapy within one year</li> <li>➤ With a family history of genetic disease or suggesting a high risk of fetal genetic disease</li> <li>➤ Maternal malignancy during pregnancy</li> </ul>

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325 Table 2 List of diseases and genes included in the screening test

Type of diseases	Diseases and genes
Aneuploidies (7 diseases)	Trisomy 21
	Trisomy 18
	Trisomy 13
	45, X
	47, XXX
	47, XXY
	47, XYY
Microdeletion and microduplication syndromes (MMS) (9 diseases)	DiGeorge syndrome
	1p36 deletion syndrome
	2q33.1 deletion syndrome
	Angelman syndrome
	Prader-Willi syndrome
	Cri du Chat syndrome
	Wolf-Hirschhorn syndrome
Monogenic disorders (155 diseases with related 75 genes)	Langer-Giedion syndrome
	Jacobsen syndrome
	Representative diseases and genes:
	Noonan spectrum disorders ( <i>PTPN11</i> , <i>SOS1</i> , <i>RIT1</i> , <i>RAF1</i> , etc.)
	Osteogenesis imperfecta ( <i>COL1A1</i> , <i>COL1A2</i> , <i>IFITM5</i> )
	Achondroplasia ( <i>FGFR3</i> )
	Crouzon syndrome ( <i>FGFR2</i> , <i>FGFR3</i> )
	CHARGE syndrome ( <i>CHD7</i> )
	Rett syndrome ( <i>MECP2</i> )
	Tuberous sclerosis ( <i>TSC1</i> , <i>TSC2</i> )

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328 Figure legends

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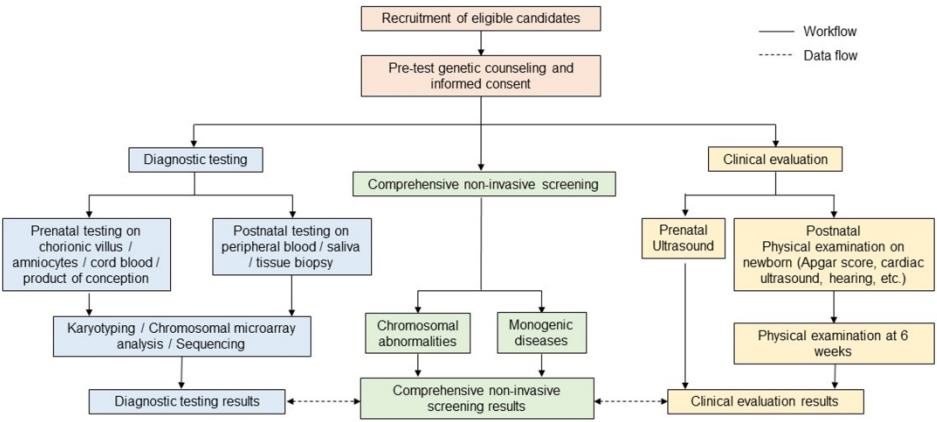
330 Figure 1 Flowchart of trial design.

331 Figure 2 Analysis plan

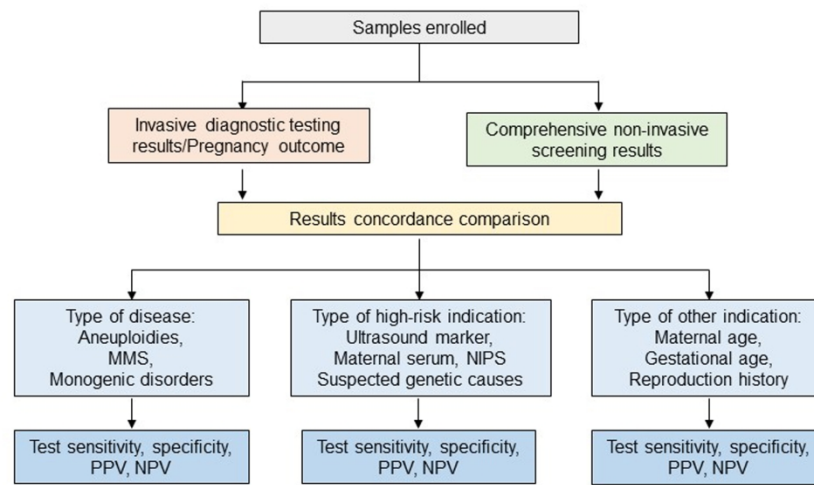
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## Comprehensive non-invasive prenatal screening for pregnancies with elevated risks of genetic disorders: protocol for a prospective, multicenter study

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# **Comprehensive non-invasive prenatal screening for pregnancies with elevated risks of genetic disorders: protocol for a prospective, multicenter study**

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ABSTRACT

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Introduction

Chromosomal abnormalities and monogenic disorders account for ~15-25% of recognizable birth defects. With limited treatment options, preconception and prenatal screening were developed to reduce the incidence of such disorders. Currently, non-invasive prenatal screening (NIPS) for common aneuploidies is implemented worldwide with superiority over conventional serum or sonographic screening approaches. However, the clinical validity for the screening of frequent chromosome segmental copy number variations (CNVs) and monogenic disorders still awaits to be proved.

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Methods and analysis

This study is a multicenter, prospective study. The participants were recruited from three tertiary hospitals in China starting from April 10<sup>th</sup>, 2021. The study is expected to conclude before October 10<sup>th</sup>, 2022. Pregnant women with abnormal prenatal screening results indicated for invasive prenatal diagnosis or those who decide to terminate their pregnancies due to abnormal ultrasound findings will be evaluated for enrollment. Cell-free DNA (cfDNA) extracted from the maternal plasma will be used for an analytically validated comprehensive NIPS test developed by Beijing BioBiggen Technology Co., Ltd. (Beijing, China). The diagnostic results from prenatal or postnatal specimens as well as the pregnancy outcome data will be collected to examine the clinical sensitivity, specificity, positive and negative predictive values of the test.

44 Ethics and dissemination

45 This study was approved by the Obstetrics and Gynecology Hospital of Fudan  
46 University (2020-178). Results of this study will be disseminated to public through  
47 scientific conferences and a peer-reviewed journal. Written informed consents will be  
48 obtained from participants.

50 Study registration number

51 ChiCTR2100045739.

## 53 STRENGTHS AND LIMITATIONS OF THIS STUDY

- 54 • This is the first prospective, multicenter clinical study for an integrated non-  
55 invasive prenatal screening test for both chromosomal abnormalities and  
56 monogenic diseases.
- 57 • This study is focused on a panel of pre-selected diseases which have relatively  
58 high incidence.
- 59 • The limitation for this study includes population stratification for high-risk  
60 pregnancies and potential loss of postnatal follow-up.

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64 INTRODUCTION

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65 Genetic etiology of birth defects

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66 Birth defects are congenital conditions causing structural or functional anomalies at

67 birth, which greatly contribute to infant mortality and disability[1]. Approximately 3–5% of

68 newborns are affected by a birth defect worldwide[2]. Although the causes of most

69 cases are unknown, about 15-25% of birth defects are due to genetic diseases such as

70 chromosomal abnormalities and monogenic disorders[3].

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72 The screening of severe genetic diseases

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73 Great efforts have been made to prevent birth defects with an underlying genetic

74 etiology. Carrier screening for recessive disorders such as Tay-Sachs disease was

75 proved to be highly effective for the reduction of its incidence[4]. The first-trimester

76 combined screening for fetal aneuploidies by prenatal ultrasound and maternal serum

77 biochemical testing detects over 85% common trisomies at a false positive rate of ~5%

78 which can lead to parental anxiety and excessive invasive diagnostic procedures for

79 otherwise normal pregnancies imposing a risk for pregnancy loss[5 6]. Since the

80 discovery of circulating fetal cfDNA in the maternal plasma during pregnancy, its

81 biological characteristics and clinical implication have been extensively studied[7 8].

82 Non-invasive evaluation for fetal gender and risks for monogenic disorders,

83 aneuploidies, and chromosome segmental CNVs were developed using different

84 molecular or genomic techniques[9-11]. Importantly, the emergence of next-generation

85 sequencing (NGS) technology enabled a practical population-based screening method

for Down syndrome [12 13]. In the past decade, NGS-based NIPS for trisomy 21, 18, and 13 has become a new standard for prenatal care with proven clinical validity [14]. Recently, NIPS was used to detect rare autosome trisomies, sex chromosome aneuploidies, and microdeletion or microduplication syndromes [15 16]. However, monogenic disorders which represent another major cause of birth defects are beyond the scope of the current screening of chromosomal abnormalities, and the clinical validity for the screening of such diseases are yet to be demonstrated.

#### The development of NIPS for monogenic disorders

Previous studies have shown that the analysis of fetal cfDNA was useful to determine the inheritance of parental alleles associated with autosomal or sex-linked recessive monogenic diseases [17 18]. Additionally, non-invasive prenatal testing was also accurate for the diagnosis or screening of the *de novo* or paternally inherited variants causing dominant diseases such as achondroplasia and Noonan spectrum disorders[19 20]. These studies showed potential clinical utility for monogenic NIPS (NIPS-M) in pregnancies at moderate risks (e.g., pregnancies with advanced paternal age or ultrasound soft markers). These tests could also be used for the screening of diseases which can only be discovered at late gestational ages (e.g., skeletal disorders).

Although the analytical accuracy of NIPS-M has been well demonstrated, such tests will not be widely accepted without further evidence-based clinical study[21]. Firstly, the clinical validity of NIPS-M has not been supported by large prospective studies. The follow-up studies of the pregnancies tested positive or negative should be evaluated by clinical examination or golden standard diagnostic tests. Additionally, although isolated

cases have demonstrated the benefits of NIPS-M leading to early diagnosis and better prenatal/postnatal management, the benefits for the management of patients with different indications are yet to be explored by larger studies[20 22 23]. Thirdly, the limitations of NIPS-M need to be evaluated. Accurate genetic counselling is critical to the success of a prenatal screening test which should provide information regarding disease characteristics, natural history, penetrance, expressivity, genotype-phenotype correlation, etc. The benefits and risks of NIPS-M need to be carefully evaluated when patients are counseled based on current understanding of the diseases[24].

This study is aimed to address above important issues with a focus on the clinical validation of an innovative NIPS for concurrent screening of common aneuploidies, CNVs and monogenic disorders. The potential benefits and the limitations of this screening test will also be explored based on the pregnancy outcome data.

## Aims

1. To assess the clinical validity of a novel NIPS test for concurrent screening of seven common aneuploidies, nine microdeletion and microduplication syndromes (MMS) and 155 monogenic disorders (75 genes).
2. To evaluate the pregnancy outcome for the participants of this comprehensive NIPS.

## METHODS AND ANALYSIS

### Study design

This prospective cohort study aims to evaluate how a comprehensive NIPS test will reveal pregnancies at risks for both chromosomal and monogenic disorders. It is a prospective, multicenter study focused on pregnant women with indications for prenatal diagnosis, including fetal ultrasound markers, high risk results by maternal serum screening or routine aneuploidy NIPS. Pregnancies with elective abortion due to fetal structural abnormality will also be recruited. To assess the performance metrics of this NIPS test, cases will be followed up to compare the screening results with the prenatal or postnatal diagnostic test results including sequencing, chromosomal CNV testing and/or karyotyping. Clinical follow-up will be pursued regarding the pregnancy outcome up to 6 weeks after birth (Figure 1).

## Consent and eligibility

Participants will be recruited from three tertiary hospitals in China including the Obstetrics and Gynecology Hospital of Fudan University (Shanghai), the Hunan Provincial Maternal and Child Health Care Hospital (Changsha), and the Women's Hospital of Zhejiang University (Hangzhou). The detailed descriptions of inclusion and exclusion criteria are shown in Table 1. Pre-test genetic counseling will be provided by healthcare providers to all participants before obtaining the written informed consent to complete enrollment. The purpose and process of this study, as well as potential benefits, risks, data privacy and rights to withdraw will be discussed during the counseling session.

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152 Patient and public involvement

153 Patients or the public were not involved in the design, conduct, or dissemination plans

154 of this research.

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156 Sample size

157 This study aims to recruit at least a total of 1,000 pregnancies undergoing invasive

158 diagnostic procedures or elective abortions due to abnormal prenatal findings

159 suggestive of severe genetic disorders. All eligible subjects will be stratified into different

160 indication groups (Table 1), and the number of subjects in each group will be

161 approximately allocated as the following: Group 1, fetal structural anomalies detected

162 by ultrasound (60%); Group 2, high risk by routine NIPS (20%); Group 3, high risk by

163 maternal serum biochemical testing (10%); Group 4, suspected genetic causes with

164 other indications (10%). For group 1, further stratification will be achieved based on the

165 gestational age, including 12-16 weeks, 17-21 weeks, and 22 weeks and above (Table

166 1). The number of subjects from each hospital will be evenly collected given equal

167 participant availability. In this study, at least 1,000 participants will be enrolled from

168 whom we expect to detect at least 25 cases affected with a targeted monogenic or

169 chromosomal disease each. This estimation is based on the detection rate among

170 pregnancies with similar indications[25-27]. The sample size in this study allows a

171 probability of 95% or above to observe a possible measuring error at the case level for

172 both the monogenic diseases and chromosomal diseases.

## 174 Screening and reporting

175 The comprehensive non-invasive prenatal screening test used in this study was  
176 developed by Beijing BioBiggen Technology Co., Ltd. (Beijing, China). This test is  
177 based on liquid-phase target enrichment followed by high read-depth NGS which can  
178 concurrently detect a panel of pre-selected diseases consisted of seven common  
179 chromosomal aneuploidies, nine MMS, and 155 monogenic disorders (Table 2 and  
180 Table S1). A total of 10 ml peripheral blood is collected from each participant and the  
181 plasma is separated through a standard two-step centrifugation process. Manufacturer  
182 protocols are used for cfDNA extraction (TIANGEN, China) and NGS library  
183 construction (Nanodigbio, China). Custom designed hybridization probes are  
184 synthesized and used for target enrichment (Integrated DNA Technologies, USA). The  
185 final DNA library is sequenced at 2x100 paired-end mode on MGISEQ-2000 sequencer  
186 (MGI, China).

187 The pathogenicity for both chromosomal and monogenic variants is evaluated according  
188 to the American College of Medical Genetics guidelines[28 29]. Only pathogenic and  
189 likely pathogenic variants are deemed positive and reportable to patients after  
190 diagnostic confirmation. For cases with chromosomal abnormalities identified in the  
191 NIPS test, karyotyping, chromosomal microarray analysis, and whole genome  
192 sequencing for copy number variation analysis are used as diagnostic tests. For cases  
193 with screening positive monogenic variants, Sanger sequencing is used as the  
194 confirmatory test. Post-test genetic counseling is provided to participants by  
195 experienced clinical geneticists regarding the interpretation of the diagnostic results, the  
196 implications of these positive findings, and potential management options.

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198     Pregnancy outcomes follow-up

199     All screening and diagnostic testing results, clinical examination results and images,  
200     and other relevant information available to us will be collected in the participants’  
201     medical records and used for statistical analysis. All cases will be followed up for  
202     pregnancy outcome including elective abortion, miscarriage, stillbirth, or live birth.  
203     Newborns will be followed up for birth records and clinical examination or diagnostic  
204     testing up to 6 weeks of age. Subjects with positive NIPS results who do not have  
205     diagnostic genetic test results are excluded from the cohort. Subjects with negative  
206     diagnostic testing results, normal results in newborn physical examination or a genetic  
207     etiology established for diseases other than those included in our screening panel are  
208     considered as negative cases.

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210     Statistical analysis

211     The diagnostic testing and pregnancy outcome results of all pregnancies will be used to  
212     compare with the results generated by our NIPS test. The outcome is the area under  
213     the receiver-operating-characteristic (ROC) curve for detection of each type of  
214     abnormalities (aneuploidies, MMS, and monogenic disorders) by NIPS in high-risk  
215     pregnancies. The ROC curve is generated by computation of test sensitivity and  
216     specificity, and confidence intervals are computed using the Clopper–Pearson method.  
217     Assay performance metrics will also be demonstrated by false positive rate, false  
218     negative rate, positive predictive value, and negative predictive value, according to each

category of abnormalities (Figure 2). Data will be analyzed with respect to different groups of indications for high-risk pregnancies, as well as pregnancies at different maternal age or gestational age.

## Study conduct

In this study, subjects' demographic and clinical exam data are collected from test requisition forms, hospital medical records and postnatal follow-up surveys. Site monitoring of source data is performed following the study monitoring plan. All patients' privacy information collected during the study will be kept strictly confidential. Study data will be held securely on paper or electronically at the Obstetrics and Gynecology Hospital of Fudan University. Data safety will be reviewed on a regular basis to identify any safety concerns or trends.

## ETHICS AND DISSEMINATION

This study was approved by the Obstetrics and Gynecology Hospital of Fudan University (2020-178). Results of this study will be disseminated to public through scientific conferences and a peer-reviewed journal. Written informed consents will be obtained from participants. Deidentified participant data such as the screening and diagnostic results along with pregnancy outcome data will be shared at the individual or aggregative level. The assay protocols, clinical study protocol, statistical analysis plan, will be shared for at least 5 years after the clinical study is completed. The data will become available within 1 year after the clinical study is completed. All essential data

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supporting the conclusion of the study as well as detailed assay protocols, analytical algorithms, and customized computational codes will be submitted for publication within 1 year after the clinical study is completed. All the disease-causing variants and the key phenotypes found in the subjects will be published or deposited at a public database. Subjects' identifiable information including their genomic sequencing data will be kept in a clinical and privacy-compliant laboratory. Non-identifiable sequencing data (e.g., individual variant data generated by locus-specific sequencing) can be provided upon request from the corresponding author (Dr. Jinglan Zhang, [jinglanzhang@foxmail.com](mailto:jinglanzhang@foxmail.com)) for at least 5 years after the clinical study is completed.

DISCUSSION

This is the first study to evaluate the clinical validity of an integrated non-invasive prenatal screening for both chromosomal abnormalities and monogenic disorders. Although NIPS for common aneuploidies has been accepted by professional societies and widely implemented around the world, NIPS for frequent chromosomal CNVs and monogenic disorders requires evidence-based investigation to prove its validity and its potential for pregnancy management. To investigate the performance of this new NIPS test, high-risk pregnancies are selected based on the routine prenatal screening results suggestive of genetic diseases and stratified into different groups. Diagnostic testing results together with pregnancy outcome data will be obtained for the clinical validation. Additionally, important practical issues around this test will be explored and discussed. For instances, the criteria for the selection of diseases, the proper indications for this test, genetic counseling and pregnancy management options will be evaluated based

on the detection rate and pregnancy outcome to weigh the benefits and risks when offering a comprehensive NIPS test for different types of diseases. It should be noted that this study is observational, and all high-risk pregnancies recruited will be counseled by clinicians following current clinical guidelines for prenatal care. Patients' decisions regarding whether to take invasive diagnostic testing or how to proceed with their ongoing pregnancy will not be intervened by the screening results of this test unless it is confirmed by diagnostic testing. Previous studies (Table S2) assessing the clinical validity of aneuploidy or chromosomal CNV prenatal screening were also observational although most of these studies were conducted at an early gestational age when different pregnancy managing options are possible [14 15 30 31]. The primary goal for this study is to assess the clinical validity of this new comprehensive NIPS test in a high-risk population in which abnormal prenatal screening results are mostly discovered at a late gestation age. Therefore, redirecting ongoing pregnancy can be challenging when the diagnostic test result is not available in time. Future study on general pregnancy population will be performed to investigate how this test may impact the prenatal or postnatal management at an early gestation age.

Overall, in this prospective, multicenter study, we will provide invaluable data to assess the clinical validity of a novel comprehensive NIPS test for the concurrent screening of chromosomal and monogenic disorders. This test has the potential to be offered as an expanded and a next-generation NIPS test for general pregnancy population.

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304 Technology Co., Ltd..

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## Contributors

JZ and H-FH conceived the study. JZ, H-FH, YW, CX and XC participated in the design of the study and drafting of the manuscript. CX, SC, QL, HX, DZ and HW participate in recruitment of participants and assessment of clinical outcomes. XC and JZ will design the statistical analysis plan and oversee statistical analysis. All authors critically reviewed and approved the manuscript for submission.

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327 of sample processing and high-throughput sequencing was supported by Beijing  
328 BioBiggen Technology Co., Ltd..

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330 Competing interests

331 XC, JZ are employees or shareholders of Beijing BioBiggen Technology Co., Ltd.. The  
332 other authors declare no conflict of interest.

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334 Patient consent for publication

335 Not required.

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337 Ethics approval

338 This study had been reviewed and approved by the Obstetrics and Gynecology Hospital  
339 of Fudan University (2020-178).

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343 Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none"> <li>➤ Adult pregnant woman (age <math>\geq 20</math> years)</li> <li>➤ Gestational age <math>\geq 12^{+0}</math> weeks</li> <li>➤ Singleton pregnancy</li> <li>➤ High-risk pregnancy with following indications               <ol style="list-style-type: none"> <li>1. Fetal structural anomalies detected on ultrasound                   <ol style="list-style-type: none"> <li>12-16 weeks                       <ol style="list-style-type: none"> <li>a. Increased nuchal translucency or cystic hygroma</li> <li>b. Cardiac structural defects</li> <li>c. Absence or hypoplasia of nasal bone</li> </ol> </li> <li>17-21 weeks                       <ol style="list-style-type: none"> <li>a. Polycystic kidney</li> <li>b. Intrauterine growth restriction</li> <li>c. Malformation of the digestive tract</li> <li>d. Ventriculomegaly</li> <li>e. Polyhydramnios</li> <li>f. Oligohydramnios</li> <li>g. Echogenic bowel</li> <li>h. Pyelic separation</li> </ol> </li> <li>22 weeks and above                       <ol style="list-style-type: none"> <li>a. Abnormal length, shape or mineralization of long bones</li> <li>b. Abnormal number of fingers and toes</li> <li>c. Abnormal shape of palms and soles</li> <li>d. Abnormal circumference of head, abdomen or chest</li> <li>e. Abnormal mineralization and shape of skull and spine</li> <li>f. Abnormal size and shape of scapula, clavicle, forehead, nasal bone or mandible</li> <li>g. Abnormal posture of limbs</li> </ol> </li> </ol> </li> <li>2. High risk by routine NIPS</li> <li>3. High risk by maternal serum biochemical testing</li> <li>4. Suspected genetic causes such as recurrent miscarriage</li> </ol> </li> <li>➤ Acceptance for a diagnostic procedure which has leftover specimens such as chorionic villus, amniotic cells, cord blood, product of conception, or newborn's peripheral blood</li> </ul>	<ul style="list-style-type: none"> <li>➤ Age <math>&lt; 20</math> years</li> <li>➤ Gestational age <math>&lt; 12^{+0}</math> weeks</li> <li>➤ Chromosomal abnormality in either of the couple</li> <li>➤ Received allogeneic blood transfusion, organ transplantation, or cell therapy within one year</li> <li>➤ Family history of a genetic disease indicated for an invasive diagnostic test</li> <li>➤ Maternal malignancy during pregnancy</li> </ul>

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344 Table 2 The list of diseases and genes included in the screening test

Type of diseases	Diseases and genes
Aneuploidies (7 diseases)	Trisomy 21
	Trisomy 18
	Trisomy 13
	45, X
	47, XXX
	47, XXY
	47, XYY
Microdeletion and microduplication syndromes (9 diseases)	DiGeorge syndrome
	1p36 deletion syndrome
	2q33.1 deletion syndrome
	Angelman syndrome
	Prader-Willi syndrome
	Cri du Chat syndrome
	Wolf-Hirschhorn syndrome
	Langer-Giedion syndrome
	Jacobsen syndrome
Monogenic disorders (155 diseases with related 75 genes)*	Representative diseases and genes: Noonan spectrum disorders ( <i>PTPN11</i> , <i>SOS1</i> , <i>RIT1</i> , <i>RAF1</i> , etc.)
	Osteogenesis imperfecta ( <i>COL1A1</i> , <i>COL1A2</i> , <i>IFITM5</i> )
	Achondroplasia ( <i>FGFR3</i> )
	Crouzon syndrome ( <i>FGFR2</i> , <i>FGFR3</i> )
	CHARGE syndrome ( <i>CHD7</i> )
	Rett syndrome ( <i>MECP2</i> )
	Tuberous sclerosis ( <i>TSC1</i> , <i>TSC2</i> )

345 \*See supplemental materials for the full list of monogenic disorders.

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347 Figure legends

348 Figure 1 The diagram for the clinical validation of a comprehensive non-invasive  
349 prenatal screening test.

350

351 Figure 2 The diagram for the screening result analyses based on different disease types  
352 and indications. MMS: microdeletion and microduplication syndromes. NIPS: non-  
353 invasive prenatal screening. PPV: positive predictive value. NPV: negative predictive  
354 value.

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For peer review only

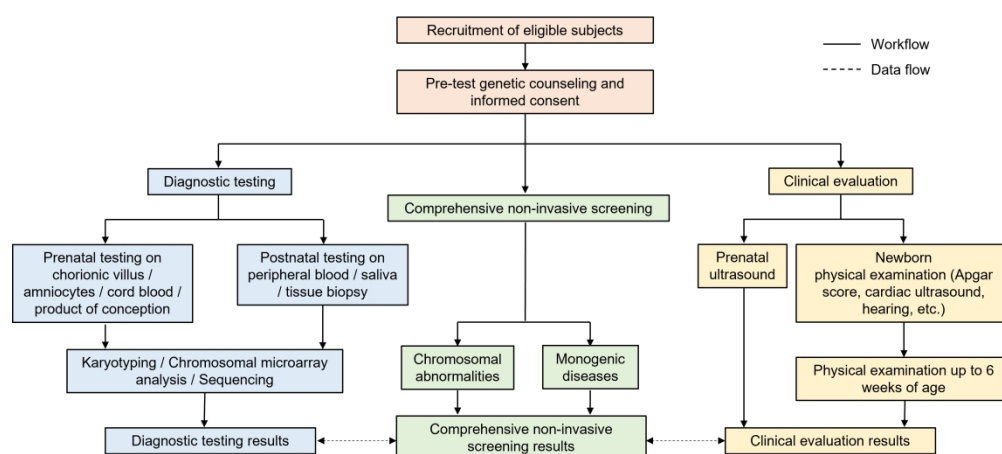


Figure 1 The diagram for the clinical validation of a comprehensive non-invasive prenatal screening test.

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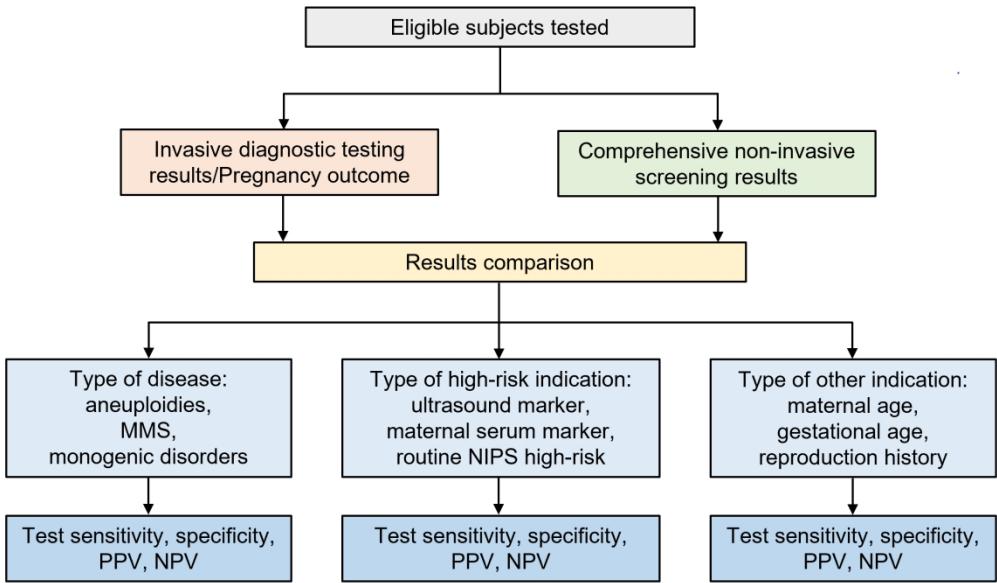


Figure 2 The diagram for the screening result analyses based on different disease types and indications. MMS: microdeletion and microduplication syndromes. NIPS: non-invasive prenatal screening. PPV: positive predictive value. NPV: negative predictive value.

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Table S1. The complete list for diseases screened in the study

Diseases	Phenotype MIM number	Inheritance	Gene
Parietal foramina 2	609597	AD	ALX4
Parietal foramina 1	168500	AD	MSX2
Parietal foramina with cleidocranial dysplasia	168550	AD	MSX2
Craniosynostosis 2	604757	AD	MSX2
Cardiofaciocutaneous syndrome 1	115150	AD	BRAF
LEOPARD syndrome 3	613707	AD	BRAF
Noonan syndrome 7	613706	AD	BRAF
Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia	613563	AD	CBL
Costello syndrome/Congenital myopathy with excess of muscle spindles	218040	AD	HRAS
Cardiofaciocutaneous syndrome 2	615278	AD	KRAS
Noonan syndrome 3	609942	AD	KRAS
RAS-associated autoimmune leukoproliferative disorder	614470	AD	KRAS
cardiofaciocutaneous syndrome 3	615279	AD	MAP2K1
cardiofaciocutaneous syndrome 4	615280	AD	MAP2K2
Noonan syndrome 6	613224	AD	NRAS
LEOPARD syndrome 1	151100	AD	PTPN11
Metachondromatosis	156250	AD	PTPN11
Noonan syndrome 1	163950	AD	PTPN11
Cardiomyopathy, dilated, 1NN	615916	AD	RAF1
Noonan syndrome 5	611553	AD	RAF1
Noonan syndrome 8	615355	AD	RIT1
Noonan syndrome-like with loose anagen hair 1	607721	AD	SHOC2
Noonan syndrome4	610733	AD	SOS1
Noonan syndrome 9	616559	AD	SOS2
Epileptic encephalopathy, early infantile, 2	300672	XLD	CDKL5
CHARGE syndrome	214800	AD	CHD7
Hypogonadotropic hypogonadism 5 with or without anosmia	612370	AD	CHD7
Metaphyseal chondrodysplasia, Schmid type	156500	AD	COL10A1
Marshall syndrome	154780	AD	COL11A1
Stickler syndrome, type II	604841	AD	COL11A1
Achondrogenesis, type II or hypochondrogenesis	200610	AD	COL2A1
Avascular necrosis of the femoral head	608805	AD	COL2A1
Czech dysplasia	609162	AD	COL2A1
Kniest dysplasia	156550	AD	COL2A1
Legg-Calve-Perthes disease	150600	AD	COL2A1
Osteoarthritis with mild chondrodysplasia	604864	AD	COL2A1
Platyspondylic skeletal dysplasia, Torrance type	151210	AD	COL2A1
SED congenita	183900	AD	COL2A1

SMED Strudwick type	184250	AD	COL2A1
Spondyloepiphyseal dysplasia, Stanescu type	616583	AD	COL2A1
Spondyloperipheral dysplasia	271700	AD	COL2A1
Stickler syndrome, type I, nonsyndromic ocular	609508	AD	COL2A1
Stickler syndrome, type I	108300	AD	COL2A1
Caffey disease	114000	AD	COL1A1
Ehlers-Danlos syndrome, arthrochalasia type, 1	130060	AD	COL1A1
Osteogenesis imperfecta, type I	166200	AD	COL1A1
Osteogenesis imperfecta, type II	166210	AD	COL1A1;COL1A2
Osteogenesis imperfecta, type III	259420	AD	COL1A1;COL1A2
Osteogenesis imperfecta, type IV	166220	AD	COL1A1;COL1A2
Ehlers-Danlos syndrome, arthrochalasia type, 2	617821	AD	COL1A2
Chondrodysplasia punctata, X-linked dominant	302960	XLD	EBP
Capillary malformation-arteriovenous malformation 2	618196	AD	EPHB4
Lymphatic malformation 7	617300	AD	EPHB4
Craniosynostosis 4	600775	AD	ERF
Chitayat syndrome	617180	AD	ERF
Acromicric dysplasia	102370	AD	FBN1
Ectopia lentis, familial	129600	AD	FBN1
Geleophysic dysplasia 2	614185	AD	FBN1
Marfan lipodystrophy syndrome	616914	AD	FBN1
Marfan syndrome	154700	AD	FBN1
MASS syndrome	604308	AD	FBN1
Stiff skin syndrome	184900	AD	FBN1
Weill-Marchesani syndrome 2, dominant	608328	AD	FBN1
Antley-Bixler syndrome without genital anomalies or disordered steroidogenesis	207410	AD	FGFR2
Apert syndrome	101200	AD	FGFR2
Beare-Stevenson cutis gyrata syndrome	123790	AD	FGFR2
Bent bone dysplasia syndrome	614592	AD	FGFR2
Crouzon syndrome	123500	AD	FGFR2
Jackson-Weiss syndrome	123150	AD	FGFR2;FGFR1
LADD syndrome	149730	AD	FGFR2;FGFR3
Pfeiffer syndrome/Craniofacial-skeletal-dermatologic dysplasia	101600	AD	FGFR2;FGFR1
Saethre-Chotzen syndrome/Saethre-Chotzen syndrome with or without eyelid anomalies	101400	AD	FGFR2
achondroplasia	100800	AD	FGFR3
Crouzon syndrome with acanthosis nigricans	612247	AD	FGFR3
hypochondroplasia	146000	AD	FGFR3
Muenke syndrome	602849	AD	FGFR3
SADDAN	616482	AD	FGFR3
thanatophoric dysplasia type I	187600	AD	FGFR3
thanatophoric dysplasia type II	187601	AD	FGFR3

Atelosteogenesis, type I	108720	AD	FLNB
Atelosteogenesis, type III	108721	AD	FLNB
Boomerang dysplasia	112310	AD	FLNB
Larsen syndrome	150250	AD	FLNB
Cornelia de Lange syndrome 1	122470	AD	NIPBL
Cornelia de Lange syndrome 2	300590	XLD	SMC1A
Cornelia de Lange syndrome 3	610759	AD	SMC3
Cornelia de Lange syndrome 4	614701	AD	RAD21
Cornelia de Lange syndrome 5	300882	XLD	HDAC8
Au-Kline syndrome	616580	AD	HNRNPK
Osteogenesis imperfecta, type V	610967	AD	IFITM5
Genitopatellar syndrome	606170	AD	KAT6B
SBBYSS syndrome	603736	AD	KAT6B
Kabuki syndrome 1	147920	AD	KMT2D
Pelger-Huet anomaly	169400	AD	LBR
Cardiomyopathy, dilated, 1A	115200	AD	LMNA
Emery-Dreifuss muscular dystrophy 2, autosomal dominant	181350	AD	LMNA
Heart-hand syndrome, Slovenian type	610140	AD	LMNA
Lipodystrophy, familial partial, type 2	151660	AD	LMNA
Malouf syndrome	212112	AD	LMNA
Muscular dystrophy, congenital	613205	AD	LMNA
Rett syndrome	312750	XLD	MECP2
Neurofibromatosis-Noonan syndrome	601321	AD	NF1
Neurofibromatosis, familial spinal	162210	AD	NF1
Neurofibromatosis, type 1	162200	AD	NF1
Watson syndrome	193520	AD	NF1
Neurofibromatosis, type 2	101000	AD	NF2
Sotos syndrome 1	117550	AD	NSD1
CHILD syndrome	308050	XLD	NSDHL
Polycystic kidney disease 1	173900	AD	PKD1
Polycystic kidney disease 2	613095	AD	PKD2
Acrodysostosis 1, with or without hormone resistance	101800	AD	PRKAR1A
Carney complex, type 1	160980	AD	PRKAR1A
Myxoma, intracardiac	255960	AD	PRKAR1A
Pigmented nodular adrenocortical disease, primary, 1	610489	AD	PRKAR1A
Failure of tooth eruption, primary	125350	AD	PTH1R
Metaphyseal chondrodysplasia, Murk Jansen type	156400	AD	PTH1R
Neurodevelopmental disorder with or without anomalies of the brain, eye, or heart	616975	AD	RERE
Cleidocranial dysplasia	119600	AD	RUNX2
Metaphyseal dysplasia with maxillary hypoplasia with or without brachydactyly	156510	AD	RUNX2
King-Denborough syndrome	145600	AD	RYR1

Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome	175050	AD	SMAD4
Myhre syndrome	139210	AD	SMAD4
Polyposis, juvenile intestinal	174900	AD	SMAD4
Aortic valve disease 2	614823	AD	SMAD6
Cerebrocostomandibular syndrome	117650	AD	SNRPB
Hyper-IgE recurrent infection syndrome	147060	AD	STAT3
Autoimmune disease, multisystem, infantile-onset, 1	615952	AD	STAT3
Cardiac, facial, and digital anomalies with developmental delay	618164	AD	TRAF7
Tuberous sclerosis-1	191100	AD	TSC1
Tuberous sclerosis-2	613254	AD	TSC2
Structural brain anomalies with impaired intellectual development and craniosynostosis	618736	AD	ZIC1
Acampomelic campomelic dysplasia/Campomelic dysplasia/Campomelic dysplasia with autosomal sex reversal	114290	AD	SOX9
Craniosynostosis 1	123100	AD	TWIST1
Robinow-Sorauf syndrome	180750	AD	TWIST1
Sweeney-Cox syndrome	617746	AD	TWIST1
Brachydactyly, type A1	112500	AD	IHH
Craniosynostosis 3	615314	AD	TCF12
Loeys-Dietz syndrome 1	609192	AD	TGFBR1
Loeys-Dietz syndrome 2	610168	AD	TGFBR2
Shprintzen-Goldberg syndrome	182212	AD	SKI
Greig cephalopolysyndactyly syndrome	175700	AD	GLI3
Pallister-Hall syndrome	146510	AD	GLI3
Polydactyly, postaxial, types A1 and B	174200	AD	GLI3
Polydactyly, preaxial, type IV	174700	AD	GLI3
C syndrome	211750	AD	CD96
Bohring-Opitz syndrome	605039	AD	ASXL1
Craniofrontonasal dysplasia	304110	XLD	EFNB1
Hartsfield syndrome	615465	AD	FGFR1
Hypogonadotropic hypogonadism 2 with or without anosmia	147950	AD	FGFR1
Osteoglophonic dysplasia	166250	AD	FGFR1
Trigonocephaly 1	190440	AD	FGFR1
Trigonocephaly 2	614485	AD	FREM1
Fontaine progeroid syndrome	612289	AD	SLC25A24
Hypertelorism, Teebi type	145420	AD	SPECC1L
Opitz GBBB syndrome, type II	145410	AD	SPECC1L

Table S2. The comparison of current and previous clinical studies for non-invasive prenatal screening

Study	PMID	Target diseases	Population studied	Nature of study	Screening method	Sample size	Positive cases	Reference method	Pregnancy outcome follow-up
Norton ME, et al. Am J Obstet Gynecol. 2012	22742782	T21, T18	high risk	multicenter, prospective, observational	chromosome selective sequencing	4,002	119	arrayotype, SH, qPCR	not performed
Nicolaides KH, et al. Am J Obstet Gynecol. 2012	23107079	T21, T18	average risk	single center, retrospective	chromosome selective sequencing	2,049	11	arrayotype	newborn examination
Bianchi DW, et al. N Engl J Med. 2014	25099587	T21, T18, T13	average risk	multicenter, prospective, observational	low-depth WGS	1,914	8	arrayotype	newborn examination
Norton ME, et al. N Engl J Med. 2015	25830321	T21, T18, T13	average risk	multicenter, prospective, observational	targeted sequencing	18,955	31	arrayotype, MA	newborn examination
Current study		7 aneuploidies, 9 MMS, 155 monogenic diseases	high risk	multicenter, prospective, observational	targeted sequencing	>1000	>50	arrayotype, MA, CNV-seq, Sanger	newborn examination