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Prospective prenatal cell-free DNA screening for genetic conditions of heterogenous etiologies

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A list of authors and their affiliations appears at the end of the paper

Prenatal cell-free DNA (cfDNA) screening uses extracellular fetal DNA circulating in the peripheral blood of pregnant women to detect prevalent fetal chromosomal anomalies. However, numerous severe conditions with underlying single-gene defects are not included in current prenatal cfDNA screening. In this prospective, multicenter and observational study, pregnant women at elevated risk for fetal genetic conditions were enrolled for a cfDNA screening test based on coordinative allele-aware target enrichment sequencing. This test encompasses the following three of the most frequent pathogenic genetic variations: aneuploidies, microdeletions and monogenic variants. The cfDNA screening results were compared to invasive prenatal or postnatal diagnostic test results for 1,090 qualified participants. The comprehensive cfDNA screening detected a genetic alteration in 135 pregnancies with 98.5% sensitivity and 99.3% specificity relative to standard diagnostics. Of 876 fetuses with suspected structural anomalies on ultrasound examination, comprehensive cfDNA screening identified 55 (56.1%) aneuploidies, 6 (6.1%) microdeletions and 37 (37.8%) single-gene pathogenic variants. The inclusion of targeted monogenic conditions alongside chromosomal aberrations led to a 60.7% increase (from 61 to 98) in the detection rate. Overall, these data provide preliminary evidence that a comprehensive cfDNA screening test can accurately identify fetal pathogenic variants at both the chromosome and single-gene levels in high-risk pregnancies through a noninvasive approach, which has the potential to improve prenatal evaluation of fetal risks for severe genetic conditions arising from heterogenous molecular etiologies. ClinicalTrials.gov registration: ChiCTR2100045739.

Birth defects are structural or functional abnormalities that can occur during intrauterine life, at birth or later in infancy[1]. In live newborns, the prevalence of birth defects is approximately 2–4%, while it is increased in spontaneous miscarriages and stillbirths[2,3]. Genetic variations derived from chromosome aberrations and single-gene variants are among the leading factors causing birth defects which account for 13–15% of their underlying etiology[4]. To ameliorate the impacts of genetic conditions on affected patients and their families, most of which have no effective treatments, carrier and newborn screening for conditions such as Tay-Sachs disease, cystic fibrosis and phenylketonuria have been implemented before or after birth[5]. These population-based screening tests have resulted in timely diagnosis, optimized treatment and overall reduced birth defect incidence[4,6–8]. Importantly, the discovery of fetal cell-free DNA (cfDNA) in pregnant women's peripheral blood elicited the development of noninvasive screening for Down syndrome (trisomy 21 or T21) and other frequent chromosomal abnormalities[9,10].

e-mail: jinglanzhang@foxmail.com; wanghua213@aliyun.com; zhangdan@zju.edu.cn; chenming_xu2006@163.com; huanghefg@hotmail.com

After clinical studies demonstrated its significantly improved accuracy over conventional maternal serum and/or image-based prenatal screening for the detection of aneuploidies, prenatal cfDNA screening has been widely adopted around the world[11–15]. With highly efficient DNA sequencing technologies and bioinformatic tools, cfDNA screening has been expanded to include microdeletion syndromes caused by chromosome segmental copy number losses such as 22q11.2 deletion syndrome[16–18]. Importantly, the fetal cfDNA analysis has enabled the clinical application of noninvasive prenatal diagnosis for single-gene conditions in high-risk pregnancies, including those with abnormal fetal ultrasound findings[19–21]. This approach has demonstrated its high degree of accuracy, thereby reducing unnecessary invasive diagnostic procedures[22–24]. Initial investigations of prenatal cfDNA screening for multiple monogenic conditions within a diverse population have indicated promising results, but meticulous follow-up studies centered on individual patient diagnostic outcomes are required to substantiate its validity in a clinical context[25–27].

In more than half of pediatric patients and fetuses with single-gene defects, these conditions are attributed to de novo monogenic variants[28–31]. However, the detection of such variants falls outside the purview of conventional prenatal cfDNA screening or parental carrier screening. As a result, with the existing standard of prenatal care, severe, monogenic conditions like *FGFR3*-related skeletal dysplasia are typically detected first through fetal ultrasound screening[32]. By this stage, the available options for managing the pregnancy may be substantially constrained. To counter these limitations, we have recently developed a new prenatal cfDNA screening technique, known as coordinative allele-aware target enrichment sequencing (COATE-seq) for the concurrent screening of monogenic and chromosomal conditions[33]. COATE-seq attenuates both intra-allelic and interallelic hybridization bias, thereby enhancing the detection of low-level fetal variants associated with common aneuploidies or copy number variations (CNVs; Extended Data Fig. 1)[33]. Furthermore, by leveraging the advantages of pair-end and high-coverage sequencing, this assay simultaneously analyzes both the cfDNA fragment length and allelic fraction associated with fetal monogenic variants. Such a dual strategy, used within a single test, results in simultaneous and enhanced detection of both chromosomal and monogenic variants[33]. Although cfDNA screening cannot replace phenotype-driven screening or diagnostic procedures executed via fetal imaging, it is complementary to existing strategies, thereby enhancing the detection rate of fetuses with genetic conditions of various molecular origins[34–41]. However, the accuracy of such a comprehensive screening approach has not yet been explored in routine clinical settings through prospective cohort studies. Additionally, it remains uncertain to what extent there is an additional detection yield when incorporating monogenic conditions beyond those chromosomal abnormalities in current methods.

Given the substantial impact of cfDNA screening in prenatal care, this prospective observational study aims to evaluate the clinical validity and detection capabilities of a new prenatal screening methodology using COATE-seq, which targets three of the most prevalent types of pathogenic genetic variants: aneuploidies, microdeletions and monogenic variants.

Results

Patients and data collection

Between 24 April 2021 and 10 September 2022, 1,191 sequentially identified pregnant women at elevated risk for fetal genetic conditions were enrolled and followed up in a prospective and observational clinical study from three maternity hospitals in different provinces of China. All participants underwent a comprehensive prenatal cfDNA screening test, which included the analysis of seven common aneuploidies, nine microdeletions and monogenic conditions associated with 75 genes (Extended Data Tables 1 and 2). A total of 101 participants were excluded from further analyses. Of these, 71 had no diagnostic test results available for fetal germline variants, 15 had maternal variants in targeted genomic regions interfering with fetal assessment, 8 did not meet the sequencing depth requirements for the screening test and in 7 cases, the sequencing data failed quality control for singleton pregnancy due to multiple gestation or sample contamination (Fig. 1). The mean maternal age of all qualified participants in the final cohort (n = 1,090) was 30.8 years (Table 1). The proportion of women carrying pregnancies at the gestational ages of 12–18 weeks, 19–24 weeks and ≥25 weeks was 28.9%, 39.8% and 31.3%, whereas the average fetal fraction for each group was 10.6%, 11.7% and 17.2%, respectively (Table 1). All pregnancies were at high risk of fetal genetic disease—876 (80.4%) had fetal ultrasound anomalies, 116 (10.6%) had abnormal maternal serum screening results, 86 (7.9%) had high-risk results in standard cfDNA screening for chromosomal conditions and 12 (1.1%) had a previous pregnancy history suggesting an increased risk for fetal genetic conditions (Table 1 and Extended Data Table 3).

Diagnostic testing outcomes, derived from invasive prenatal or postnatal procedures that are part of the standard of care, were gathered following the cfDNA screening test from a total of 1,090 participants. A total of 978 pregnant women underwent invasive prenatal procedures such as amniocentesis or chorionic villus sampling, and an additional 112 participants were tested on the products of conception or fetal cord blood (Table 1). This allowed for a comparative analysis between the results derived from the cfDNA screening and diagnostic testing (Table 2). The diagnostic tests included next-generation sequencing (NGS) single-gene panels for targeted monogenic conditions, whole-exome sequencing (WES), Sanger sequencing, chromosome microarray analysis, CNV sequencing (CNV-seq) and karyotyping (Table 3 and Extended Data Tables 4–6). All clinical pregnancy management decisions were based on the results of diagnostic testing, rather than the comprehensive cfDNA screening results, in accordance with current standard practice guidelines. Pregnancy outcomes by postnatal follow-up were pursued after which all the clinical examination results were evaluated to examine if they were consistent with the genetic diagnosis (Table 3 and Extended Data Tables 4 and 5).

The clinical validity of the comprehensive prenatal cfDNA screening

In all participants in the final cohort (n = 1,090), pathogenic genetic variants were detected in 135 (12.4%) pregnancies by the comprehensive cfDNA screening and confirmed by diagnostic testing, which included 89 aneuploidies, 9 microdeletions and 37 monogenic variants (Table 2). There were 44 trisomy 21 (T21), 12 trisomy 18 (T18), 5 trisomy 13 (T13), 15 45X, 5 47XYY, 6 47XXX and 2 47XXY fetuses with aneuploidies. The microdeletions detected included six 22q11.2del and three 4p16del cases (Fig. 1). In fetuses affected by monogenic conditions, diagnostic variants were found in the following genes (the number of affected fetuses is indicated): *FGFR3* (13), *COL2A1* (4), *PTPN11* (3), *HRAS* (2), *FGFR2* (2), *KMT2D* (2), *COL1A2* (2), *SOS1* (1), *EBP* (1), *EPHB4* (1), *SMAD4* (1), *TSC2* (1), *KRAS* (1), *COL1A1* (1), *NSD1* (1) and *NRAS* (1; Fig. 1 and Table 3). With respect to testing indication, the abovementioned diagnostic genetic variants were identified in 98 (11.2%) of 876 pregnancies with fetal structural abnormalities, 35 (40.7%) of 86 pregnancies with high-risk results on standard cfDNA screening for chromosomal conditions, 2 (1.7%) of 116 pregnancies with high-risk results on maternal serum screening and none were identified in the remaining 12 cases with previous pregnancy history suggestive of an increased risk for genetic conditions (Extended Data Table 3). Overall, the comprehensive cfDNA screening demonstrated a clinical sensitivity of 98.5% (95% confidence interval (CI), 94.3–99.7%) and specificity of 99.3% (95% CI, 98.4–99.7%) for all conditions screened (Table 2). These values were determined by comparing the screening with gold-standard diagnostic tests for all the conditions screened (Table 3 and Extended Data Tables 4, 5 and 6). The positive predictive value (PPV) and negative predictive value (NPV) were 95.7% (95% CI, 90.6–98.3%) and 99.8%

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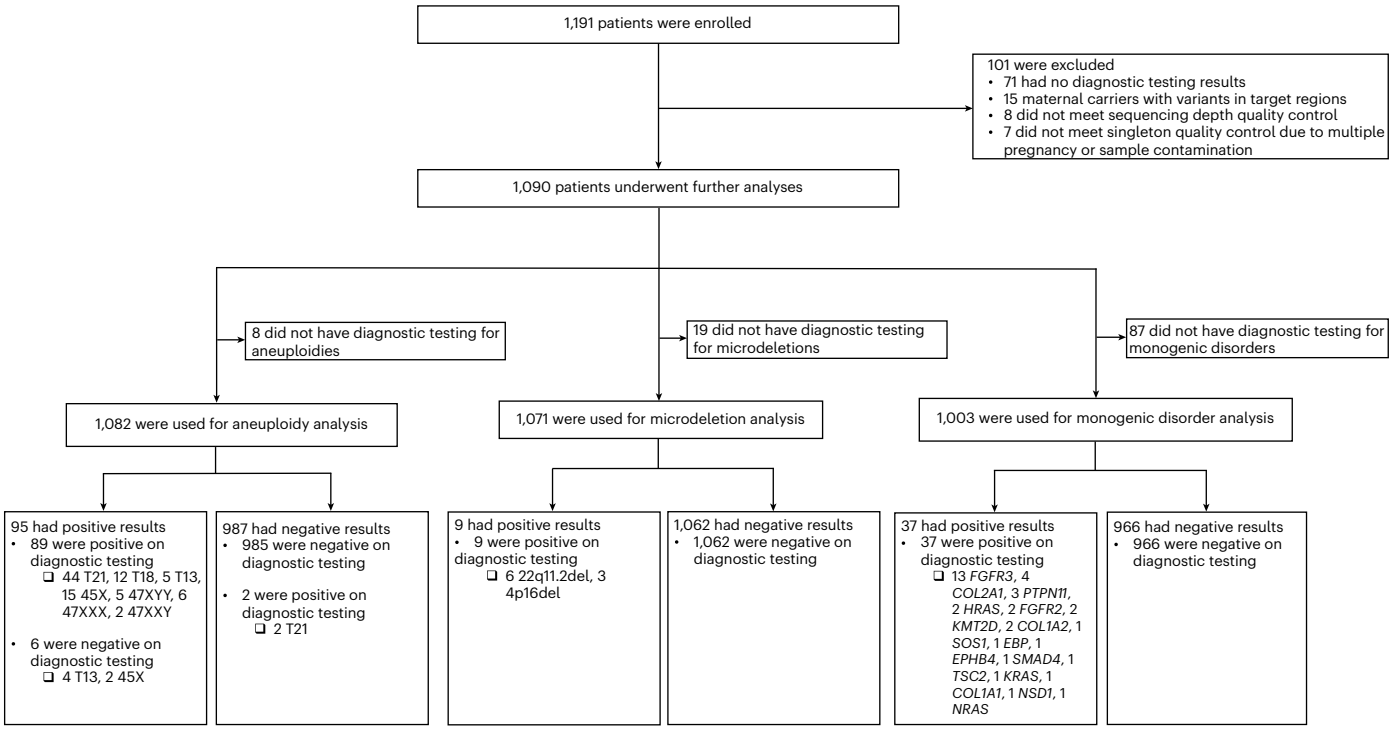

Fig. 1 | Clinical study of comprehensive prenatal cfDNA screening targeting multiple types of genetic conditions. A total of 1,191 pregnant women were enrolled. Among them, 101 were excluded including 71 without diagnostic testing results, 15 maternal carriers with variants in target regions, 8 failing sequencing depth quality control requirements and 7 failing singleton quality control requirements due to multiple pregnancies or sample contamination. A final cohort of 1,090 cases was subjected to further analysis, and 135 pregnancies were identified through the new screening method, including 89 aneuploidies, 9 microdeletions and 37 cases with monogenic conditions.

(95% CI, 99.1–100%) respectively. For aneuploidies, microdeletions, and monogenic conditions, the test sensitivity was 97.8% (95% CI, 91.5–99.6%), 100% (95% CI, 62.9–100%), and 100% (95% CI, 88.3–100%), while the test specificity was 99.4% (95% CI, 98.6–99.8%), 100% (95% CI, 99.6–100%), and 100% (95% CI, 99.5–100%), respectively (Table 2). The area under the receiver operating-characteristic (ROC) curve (AUC) for aneuploidies, microdeletions and monogenic conditions were 0.996, 1.000 and 1.000, respectively (Table 2).

There were six false-positive cases on the comprehensive cfDNA screening that yielded negative results on diagnostic tests (Extended Data Table 5). All of these pregnancies also tested positive on standard cfDNA screening using a different analytical methodology involving low-depth whole-genome sequencing (Extended Data Table 5). In addition, there were two false-negative T21 cases (with positive results on diagnostic tests) that also tested negative on standard cfDNA screening (Extended Data Table 5). Given that two different methods both yielded false screening results for the abovementioned eight cases, it was unlikely that they were caused by analytical pitfalls in the cfDNA screening but may rather be the results of the genetic differences between the fetus and placenta. Confined placenta mosaicism and restricted variants in fetuses that are absent in the placenta are known factors to cause discrepant results in prenatal cfDNA screening and diagnostic testing[42,43]. Notably, although the comprehensive cfDNA screening test produced incorrect chromosomal results for eight pregnancies, there were not any false results in all 37 positive and 966 negative cases for the monogenic conditions screened in this study (Table 2 and Fig. 1).

The detection yield of the comprehensive prenatal cfDNA screening in fetal structural anomalies

A diagnostic genetic variant was detected in 98 of 876 (11.2%) fetuses (P1–P98) with structural anomalies detected by ultrasound screening (Table 3 and Extended Data Table 4). Among them, 42 (42.9%) had common autosome aneuploidies (T21, T18 and T13), 13 (13.3%) had sex chromosome aneuploidies, 6 had microdeletions (6.1%) and 37 (37.8%) had monogenic conditions (Fig. 2a, Table 3 and Extended Data Table 4). The overall detection rate for a diagnostic genetic variant was highest in lymphatic or effusion anomalies (36.9%), followed by skeletal (24.7%) and multisystem anomalies (23.3%; Fig. 2b). The detection rate for chromosomal aberrations, including both aneuploidies and microdeletions, was highest in lymphatic or effusion abnormalities (32.6%), followed by multisystem anomalies (19.2%), increased nuchal translucency (8.8%), cardiac defects (5.7%) and craniofacial abnormalities (5.7%; Fig. 2b and Extended Data Table 3). The diagnostic yield for monogenic conditions was highest in skeletal abnormalities (23.5%), followed by lymphatic or effusion abnormalities (4.3%), multisystem anomalies (4.1%), fetal growth restriction (2.9%) and brain abnormalities (2.2%; Fig. 2b and Extended Data Table 3). The detection rate of a diagnostic genetic variant differed considerably with respect to fetal phenotypes and the underlying genetic etiologies. For instance, 32.6% of fetuses with lymphatic or effusion abnormalities had chromosomal conditions, while only 4.3% of such cases were caused by single-gene conditions (Fig. 2b and Extended Data Table 3). On the other hand, 23.5% of fetuses with skeletal anomalies were found to have monogenic conditions, while only 1.2% of such cases were attributed to chromosomal abnormalities (Fig. 2b and Extended Data Table 3).

In 13 (35.1%) of the 37 fetuses with structural anomalies caused by monogenic conditions (P18, P19, P20, P21, P22, P25, P26, P29, P32, P33, P34, P36 and P37), pathogenic variants were found in *PTPN11*, *HRAS*, *KMT2D*, *SOS1*, *SMAD4*, *TSC2*, *KRAS*, *NSD1* and *NRAS* (Table 3). Defects in these genes are known to cause postnatal neurological deficits such as learning disabilities, development delay

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Table 1 | Demographic and clinical characteristics

Characteristics	Values
Number of patients analyzed	1,090
Mean maternal age—year (range)	30.8 (20–46)
Median maternal age—year (range)	31.0 (20–46)
Mothers ≥35 years old—no. (%)	225 (20.6)
Mean maternal weight—kg (range)	60.7 (39–148)
Median maternal weight—kg (range)	59.7 (39–148)
Mean body mass index (range)	23.6 (15.4–61.4)
Mean gestational age at sample collection—week (range)	22.5 (12–37.1)
Number of pregnancies 12–18 weeks (%); mean fetal fraction (%)	315 (28.9); 10.6
Number of pregnancies 19–24 weeks (%); mean fetal fraction (%)	434 (39.8); 11.7
Number of pregnancies ≥25 weeks (%); mean fetal fraction (%)	341 (31.3); 17.2
Pregnancies with fetal structural anomalies	876 (80.4)
Cardiac—no. (%)	174 (19.9)
Increased nuchal translucency—no. (%)	159 (18.2)
Renal—no. (%)	108 (12.3)
Brain—no. (%)	91 (10.4)
Skeletal—no. (%)	85 (9.7)
Multisystem anomalies—no. (%)	73 (8.3)
Lymphatic or effusion—no. (%)	46 (5.3)
Abdominal—no. (%)	41 (4.7)
Craniofacial—no. (%)	35 (4.0)
Fetal growth restriction—no. (%)	35 (4.0)
Chest—no. (%)	17 (1.9)
Spinal—no. (%)	12 (1.4)
Pregnancies with high-risk results on standard prenatal cfDNA screening—no. (%)	86 (7.9)
Pregnancies with high-risk results on maternal serum screening—no. (%)	116 (10.6)
Pregnancies with positive clinical history (for example, recurrent miscarriage)—no. (%)	12 (1.1)
Diagnostic testing on amniocytes—no. (%)	977 (89.6)
Diagnostic testing on chorionic villus—no. (%)	1 (0.1)
Diagnostic testing on product of conception—no. (%)	111 (10.2)
Diagnostic testing on umbilical cord blood—no. (%)	1 (0.1)
Pregnancy outcome live birth—no. (%)	623 (57.2)
Pregnancy outcome elective abortion—no. (%)	268 (24.6)
Pregnancy outcome spontaneous abortion (%)	2 (0.2)
Unknown pregnancy outcome—no. (%)	197 (18.1)

Table 2 | Clinical performance of the comprehensive prenatal cfDNA screening

Parameters	Results
Overall	
True positive	135
True negative	865
False positive	6
False negative	2
Sensitivity (95% CI)	98.5% (94.3–99.7%)
Specificity (95% CI)	99.3% (98.4–99.7%)
Accuracy (95% CI)	99.2% (98.4–99.6%)
PPV (95% CI)	95.7% (90.6–98.3%)
NPV (95% CI)	99.8% (99.1–100%)
Aneuploidies	
True positive	89
True negative	985
False positive	6
False negative	2
Sensitivity (95% CI)	97.8% (91.5–99.6%)
Specificity (95% CI)	99.4% (98.6–99.8%)
Accuracy (95% CI)	99.3% (98.5–99.7%)
PPV (95% CI)	93.7% (86.2–97.4%)
NPV (95% CI)	99.8% (99.2–100%)
AUC	0.996
Microdeletions	
True positive	9
True negative	1,062
False positive	0
False negative	0
Sensitivity (95% CI)	100% (62.9–100%)
Specificity (95% CI)	100% (99.6–100%)
Accuracy (95% CI)	100% (99.6–100%)
PPV (95% CI)	100% (62.9–100%)
NPV (95% CI)	100% (99.6–100%)
AUC	1.000
Monogenic conditions	
True positive	37
True negative	966
False positive	0
False negative	0
Sensitivity (95% CI)	100% (88.3–100%)
Specificity (95% CI)	100% (99.5–100%)
Accuracy (95% CI)	100% (99.5–100%)
PPV (95% CI)	100% (88.3–100%)
NPV (95% CI)	100% (99.5–100%)
AUC	1.000

The overall test sensitivity and specificity were calculated based on all confirmed positive cases through diagnostic testing on the variant detected by cfDNA screening and all negative cases confirmed by diagnostic tests covering all three types of variants

and intellectual impairment, even though the affected fetuses did not show substantial central nervous system anomalies on routine prenatal ultrasound screening (Table 3). Overall, the detection for a diagnostic genetic variant was increased by 60.7% (from 61 to 98) for pregnancies with fetal structural anomalies when those targeted monogenic conditions were analyzed in conjunction with chromosomal conditions (Fig. 2a and Extended Data Table 3). Note that the monogenic conditions associated with the 75 genes were selected specifically for this high-risk cohort. For an extended population, more stringent criteria should be applied, focusing on genes related to conditions characterized by severe outcomes, early onset, prevalent incidence and high analytical performance (Extended Data Table 2).

Table 3 | Summary of fetuses affected by monogenic conditions identified by comprehensive prenatal cfDNA screening and confirmed by diagnostic testing

Participants	GA (weeks)	MA (years)	Indications	FF (%)	Comprehensive prenatal cfDNA screening results	Diagnostic testing and pregnancy outcomes
P1	18.7	27	Systemic skeletal malformations	7.1	*FGFR3* (NM_000142.4): c.742C>T, p.Arg248Cys, thanatophoric dysplasia	Amniocentesis; WES; elective abortion
P2	22.0	27	Generalized skeletal dysplasia, narrow aortic diameter, small cerebellum	12.9	*FGFR3* (NM_000142.4): c.746C>G, p.Ser249Cys, thanatophoric dysplasia	Product of conception; NGS-SGD and CNV-seq; elective abortion
P3	23.0	29	Systemic skeletal malformations	7.6	*FGFR3* (NM_000142.4): c.1118A>G, p.Tyr373Cys, thanatophoric dysplasia	Product of conception; Sanger and CNV-seq; elective abortion
P4	27.9	31	Short long bones	16.9	*FGFR3* (NM_000142.4): c.1138G>A, p.Gly380Arg, achondroplasia	Product of conception; NGS-SGD and CNV-seq; elective abortion
P5	21.7	32	NF 6.2 mm, enlarged head, shortened femur, humerus, and fibula	14.0	*FGFR3* (NM_000142.4): c.1138G>A, p.Gly380Arg, achondroplasia	Amniocentesis; NGS-SGD; elective abortion
P6	30.9	34	Shortened femur and humerus	22.5	*FGFR3* (NM_000142.4): c.1138G>A, p.Gly380Arg, achondroplasia	Product of conception; Sanger and NGS-SGD; elective abortion
P7	28.0	29	Shortened femur and humerus	17.4	*FGFR3* (NM_000142.4): c.1138G>A, p.Gly380Arg, achondroplasia	Product of conception; Sanger and NGS-SGD; elective abortion
P8	31.9	29	Shortened femur and humerus	30.2	*FGFR3* (NM_000142.4): c.1138G>A, p.Gly380Arg, achondroplasia	Amniocentesis; WES
P9	30.9	29	Growth restriction	19.5	*FGFR3* (NM_000142.4): c.1138G>A, p.Gly380Arg, achondroplasia	Cord blood; WES; elective abortion
P10	20.6	30	Skeletal dysplasia, hydrocephalus	18.1	*FGFR3* (NM_000142.4): c.1948A>G, p.Lys650Glu, Thanatophoric dysplasia	Product of conception; NGS-SGD; elective abortion
P11	16.0	37	Skeletal dysplasia	11.5	*FGFR3* (NM_000142.4): c.1948A>G, p.Lys650Glu, thanatophoric dysplasia	Product of conception; NGS-SGD; elective abortion
P12	16.4	30	Short limbs and narrow thorax	8.2	*FGFR3* (NM_000142.4): c.2421A>T, p.*807Cysext*101, thanatophoric dysplasia	Amniocentesis; WES and NGS-SGD; elective abortion
P13	18.0	36	Short limbs	9.3	*FGFR3* (NM_000142.4): c.2419T>G, p.(*807Glyext*101), thanatophoric dysplasia	Amniocentesis; NGS-SGD; elective abortion
P14	24.3	26	Skeletal dysplasia, micrognathia, short long bones	19.3	*COL2A1* (NM_001844.5): c.1546G>A, p.Gly516Ser, achondrogenesis	Amniocentesis; WES and NGS-SGD; elective abortion
P15	13.0	32	Nuchal translucency 7.4 mm, micrognathia, abnormal heart structures	15.1	*COL2A1* (NM_001844.5): c.1597C>T, p. Arg533*, hypochondrogenesis	Amniocentesis; NGS-SGD; elective abortion
P16	15.0	26	Skeletal dysplasia, NF thickening	5.4	*COL2A1* (NM_001844.5): c.2887G>A, p.Gly963Ser, achondrogenesis	Product of conception; WES and NGS-SGD; elective abortion
P17	13.1	34	Encephalocele, extremely short limbs, single umbilical artery	9.3	*COL2A1* (NM_001844.5):c.2951G>A, p.Gly984Asp, achondrogenesis	Amniocentesis; Sanger and NGS-SGD; elective abortion
P18	18.6	32	Venous catheter absent	6.8	*PTPN11* (NM_002834.4): c.844A>G, p.Ile282Val, Noonan spectrum disorder	Amniocentesis; NGS-SGD; elective abortion
P19	18.1	35	Nuchal translucency 4.5 mm	6.8	*PTPN11* (NM_002834.4): c.1510A>G, p.Met504Val, Noonan spectrum disorder	Amniocentesis; NGS-SGD; liveborn
P20	23.3	27	NF 8.1 mm	7.9	*PTPN11* (NM_002834.4): c.1510A>G, p.Met504Val, Noonan spectrum disorder	Amniocentesis; NGS-SGD
P21	18.1	28	NF 6.0 mm, cystic hygroma	10.9	*HRAS* (NM_005343.4): c.34G>A, p.Gly12Ser, Costello syndrome	Amniocentesis; NGS-SGD
P22	18.1	34	Single umbilical artery, cystic hygroma	17.1	*HRAS* (NM_005343.4): c.38G>A, p.Gly13Asp, Costello syndrome	Product of conception; NGS-SGD; elective abortion
P23	24.1	31	Syndactyly	17.3	*FGFR2* (NM_000141.4): c.755C>G, p.Ser252Trp, Apert syndrome	Product of conception; WES; elective abortion
P24	23.4	34	Lateral ventriculomegaly	12.0	*FGFR2* (NM_000141.4): c.1025G>C, p.Cys342Ser, Pfeiffer syndrome	Amniocentesis; NGS-SGD; liveborn
P25	17.0	32	Left ventricular hypoplasia, right ventricle double outlet	8.9	*KMT2D* (NM_003482.3): c.2263dup, p.R755Pfs*3, Kabuki syndrome	Amniocentesis; Sanger and NGS-SGD; elective abortion
P26	23.6	34	Small fetus, multiple abnormalities	3.1	*KMT2D* (NM_003482.3): c.8453G>A, p.Trp2818Ter, Kabuki syndrome	Amniocentesis, NGS-SGD; elective abortion

Table 3 (continued) | Summary of fetuses affected by monogenic conditions identified by comprehensive prenatal cfDNA screening and confirmed by diagnostic testing

Participants	GA (weeks)	MA (years)	Indications	FF (%)	Comprehensive prenatal cfDNA screening results	Diagnostic testing and pregnancy outcomes
P27	24.3	31	Shortened femur, fibula, tibia, and humerus	9.0	*COL1A2* (NM_000089.3): c.2835+1G>A, osteogenesis imperfecta	Amniocentesis; Sanger and WES; elective abortion
P28	28.6	27	Curved and short femur	13.1	*COL1A2* (NM_000089.3): c.3106G>C, p.Gly1036Arg, osteogenesis imperfecta	Amniocentesis; NGS-SGD; liveborn
P29	31.0	31	Enlarged head circumference, short long bones, dilated left renal pelvis, polyhydramnios	31.0	*SOS1* (NM_005633.3): c.1294T>C, p.Trp432Arg, Noonan spectrum disorder	Amniocentesis; WES, and NGS-SGD; elective abortion
P30	29.0	31	Spinal abnormalities, skeletal dysplasia	17.5	*EBP* (NM_006579.3): c.187C>T, p.Arg63Ter, chondrodysplasia punctata	Product of conception; NGS-SGD; elective abortion
P31	25.0	34	Single umbilical artery, pelvic ectopic kidney	14.6	*EPHB4* (NM_004444.5): c.1124dupG, p.D376Rfs*, capillary malformation-arteriovenous malformation syndrome	Amniocentesis; Sanger; elective abortion
P32	21.0	32	Lateral ventriculomegaly	4.6	*SMAD4* (NM_005359.5): c.1486C>T, p.Arg496Cys, Myhre syndrome	Amniocentesis; Sanger and NGS-SGD; elective abortion
P33	26.1	33	Cardiac rhabdomyoma	19.4	*TSC2* (NM_000548.5): c.2098-2A>G, tuberous sclerosis	Product of conception; WES; elective abortion
P34	19.6	32	Nuchal translucency 4.3 mm	8.9	*KRAS* (NM_004985.5): c.458A>T, p.Asp153Val, Noonan spectrum disorder	Amniocentesis; NGS-SGD; liveborn
P35	24.6	29	Skeletal dysplasia	10.9	*COL1A1* (NM_000088.3): c.1571G>C, p.Gly524Ala, osteogenesis imperfecta	Product of conception; WES; elective abortion
P36	26.1	33	Bilateral hydronephrosis	10.4	*NSD1* (NM_022455.4): c.7239dupT, p.Leu2414Ffs*, Sotos syndrome	Amniocentesis; NGS-SGD
P37	23.7	29	NF 19 mm, peritoneal effusion	14.9	*NRAS* (NM_002524.5): c.182A>C, p.Gln61Pro, Noonan spectrum disorder	Amniocentesis; WES, NGS-SGD; elective abortion

FF, fetal fraction; GA, gestational age (weeks); MA, maternal age (years); NF, nuchal fold; NGS-SGD, a next-generation sequencing panel test for the targeted 75 genes included in the cfDNA screening.

Pregnancy outcome for the participants undergoing the comprehensive prenatal cfDNA screening

The pregnancy outcome data were pursued up to 6 weeks after the expected delivery date. In all 1,090 qualified participants who underwent both comprehensive cfDNA screening and diagnostic test procedures, there were 623 (57.2%) live births, 268 (24.6%) elective abortions and 2 (0.2%) spontaneous abortions (Table 1). Of the total participants, 197 (18.1%) had no available pregnancy outcome data. They were enrolled and had prenatal diagnoses at one of the participating hospitals, but they sought postdiagnosis management and/or delivery at other clinical care centers (Table 1). In those 137 cases with positive results on diagnostic testing, 11 (8.0%) had live births, 106 (77.4%) had elective abortions, 1 (0.7%) had spontaneous abortion and 19 (13.9%) had unknown pregnancy outcomes (Extended Data Table 7). Among them, 100 had fetal anomalies on ultrasound screening, of which 4 (4.0%) had live births, 82 (82.0%) had elective abortions and 13 (13.0%) had unknown pregnancy outcomes (Extended Data Table 7). Pregnancy outcomes together with all postnatal and/or prenatal clinical examinations were evaluated, and no discrepancies were found between the genetic diagnosis and clinical examination (Table 3 and Extended Data Table 4). All clinical pregnancy management decisions were based on the results of diagnostic testing. In all cases with pregnancy outcome data, no adverse events were reported associated with the performing of the cfDNA screening or diagnostic tests.

The parental age effects on different types of genetic conditions

It is known that increased maternal age is one of the most substantial risk factors for fetal aneuploidies such as T21 and T18 (refs. 44,45). Advanced paternal age is associated with an increased risk for dominant conditions caused by de novo variants in single genes, such as *FGFR2*, *FGFR3* and *PTPN11* (refs. 46,47). No significant association of increased maternal or paternal age with the incidence of chromosome segmental CNV was observed[48]. In this cohort, we investigated whether parental ages were associated with the occurrence of different types of genetic conditions. In 61 pregnancies affected by autosome aneuploidies, the mean maternal age was 32.8 years, which was significantly elevated from that of 1,015 participants (30.7 years, $P = 0.005$) with no fetal autosome aneuploidy detected. The parental ages were not significantly different between the positive and negative cases for sex chromosome aneuploidies, microdeletions and monogenic conditions (Extended Data Table 8).

Discussion

In this cohort of pregnancies with elevated risks for fetal genetic conditions, we show that a comprehensive fetal cfDNA analysis can reliably identify fetuses at risks of different genetic etiologies including aneuploidies, microdeletions and monogenic conditions. The strength of this study was the use of a state-of-the-art prenatal cfDNA screening method, which concurrently detected genetic aberrations ranging from a single-nucleotide variant to whole chromosome copy number change. This method has the benefit to circumvent the typical stratification of the referral prenatal population caused by sequential testing of chromosomal and monogenic conditions, thus allowing an unbiased assessment for the detection yield of different genetic etiologies in an at-risk population. Compared to current standard screening only targeting chromosomal abnormalities, the detection rate for a diagnostic genetic variant was increased by 60.7% in the comprehensive cfDNA

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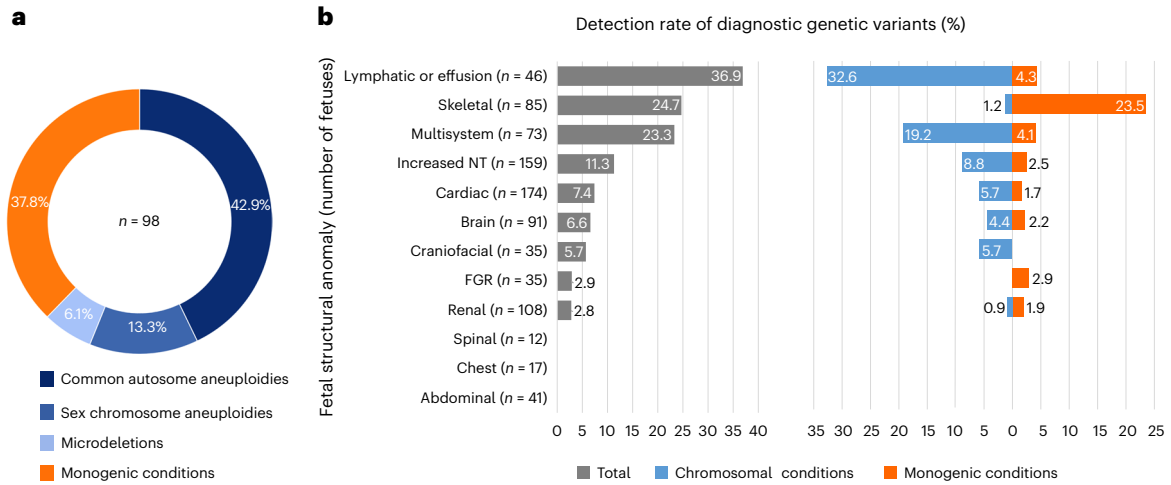

Fig. 2 | The detection rate of diagnostic genetic variants in pregnancies with fetal structural anomalies. a, A diagnostic genetic variant was detected in 98 of 876 (11.2%) pregnancies with fetal structural anomalies. Among them, 42 (42.9%) had common autosome aneuploidies, 13 (13.3%) had sex chromosome aneuploidies, 6 had microdeletions (6.1%) and 37 (37.8%) had monogenic conditions. **b**, In all 876 pregnancies with fetal structural anomalies, the detection rate for a diagnostic genetic variant was highest in lymphatic or effusion anomalies (36.9%), followed by skeletal anomalies (24.7%) and multisystem anomalies (23.3%). The detection rate for chromosomal conditions was highest in lymphatic or effusion anomalies (32.6%), followed by multisystem anomalies (19.2%), increased NT (8.8%), cardiac defects (5.7%) and craniofacial anomalies (5.7%). The detection rate for monogenic conditions was highest in skeletal anomalies (23.5%), followed by lymphatic or effusion anomalies (4.3%), multisystem anomalies (4.1%), FGR (2.9%) and brain anomalies (2.2%). NT, nuchal translucency; FGR, fetal growth restriction.

screening. Because the patient cohort included a large variety of fetal anomalies instead of targeted conditions, this study was made more generalizable to uncover the detectability of cfDNA screening for both chromosomal and monogenic variants.

The inclusion of single-gene conditions in fetal cfDNA screening has benefits for prenatal diagnosis. While such screening can never replace image-based screening procedures, it may function as an adjunctive instrument for early identification of presymptomatic fetuses during the first trimester, such as those affected by achondroplasia. In addition, some monogenic conditions are characterized by neurological defects that may not be evident on routine prenatal ultrasound screening. In the study, pathogenic variants (scored according to the American College of Medical Genetics and Genomics sequence variant interpretation guidelines) associated with postnatal neurological impairments such as learning disabilities, developmental delay and intellectual disability were identified in 13 of 37 fetuses (35.1%) with monogenic conditions who displayed no prenatal abnormalities in the brain or central nervous system. Prenatal and perinatal management can also substantially benefit from prenatal cfDNA screening, as demonstrated by a previous report on a fetus affected by Costello syndrome[49]. This becomes particularly relevant when pregnant women, aware of fetal anomalies, opt to continue their pregnancies and decline invasive procedures. In these situations, prenatal cfDNA analysis serves as an invaluable tool to guide delivery plans addressing potential neonatal complications linked to the relevant monogenic condition[49].

This study had the limitation of focusing on cfDNA screening tests in pregnancies already identified as being at elevated risk for fetal genetic conditions. This approach is advantageous for enriching the cohort with affected fetuses, thereby facilitating an effective evaluation of the test's overall sensitivity, a key parameter for screening tests. However, it leaves the performance of the test in a general obstetric population unexamined. The prior risk in the general population is expected to be significantly lower than in this high-risk cohort, a factor that could substantially impact the PPVs of the test, particularly for ultra-rare genetic conditions. It should be noted that false-positive prenatal cfDNA screening results are not uncommon for chromosomal anomalies, but its performance on dominant monogenic conditions exhibits an elevation in accuracy, at least for high-risk pregnancies[23]. Consistent with this, we have not observed false results for monogenic conditions in this cohort, in which all 37 positive findings in single genes and 966 negative cases were confirmed by diagnostic testing. Conversely, this study identified eight cases that yielded false results for aneuploidies, most likely attributable to confined placental mosaicism or divergent genomic content between the fetus and the placenta (Extended Data Table 5). Again, these observations and resultant generalizations require further validation through larger-scale cohort studies in a broader population.

Although the PPVs were reasonably high for monogenic conditions in this study, interpreting its NPVs necessitates a heightened level of scrutiny. Some monogenic conditions, caused by analytically difficult variants other than simplex short sequence variants (for example, exonic CNVs, large indels and variants obscured by homologous or repeat sequences), might elude detection using standard sequencing techniques. Consequently, the clinical NPVs for certain single-gene conditions (for example, those caused by pathogenic variants in *PKD1*) examined in this study may have been inadvertently overestimated. Future investigations using locus-specific analytical methods may assist in further refining these clinical NPVs, particularly for genes anticipated to underperform (Extended Data Table 2). Irrespective of the analytical tools used in the cfDNA assay, comprehensive pretest genetic counseling remains essential to clarify for patients that this test is a screening, not a definitive diagnostic procedure. Given that the screening is performed on fetal cfDNA originating from the placenta rather than the fetus itself, a small, yet distinct possibility persists of carrying an affected fetus even if screening test results are negative.

This study was observational in design to minimize potential adverse effects on pregnancy management, similar to previous investigations that assessed the clinical validity of prenatal cfDNA screening on targeted conditions[14,15]. Unlike typical prenatal cfDNA screenings that aim for prompt results to guide further invasive diagnostic testing, this study reported the cfDNA screening results after they were confirmed by diagnostic tests. These diagnostic tests were initiated based solely on other clinical indications (for example, fetal ultrasound abnormalities) as part of the standard of care. As a result, the cfDNA screening in this study is expected to have minimal impact on clinical decision-making in pregnancy management. Future interventional

studies shall examine the implications of this screening test on both prenatal and postnatal care, particularly when it is administered during early gestational periods with rapid result turnaround.

Prenatal cfDNA screening for chromosomal abnormalities (for example, Down syndrome) was historically offered to pregnant women of advanced maternal age (≥35 years old), but current guidelines recommend it to all pregnancies irrespective of the mother's age[50]. The correlation between the elevated occurrence of de novo single-gene variants and increased paternal age has also been well demonstrated[46,47]. Further research in larger populations is necessary before advocating the broad application of a comprehensive prenatal cfDNA screening covering monogenic conditions to pregnancies involving older parents or indeed to all pregnancies.

The main goal of this study was to evaluate a comprehensive screening method within high-risk pregnancies and ascertain the clinical validity and increased detection rate for multiple types of genetic variants relative to conventional methods. It is noteworthy that a large proportion of the pregnant women involved in this study were inclined to accept the comprehensive screening, influenced by their awareness of abnormal fetal findings. Because monogenic conditions can have extreme phenotypic and genetic heterogeneity, it is vital to exercise caution when extending an expanded screening to the general obstetrical population. Therefore, it is imperative to establish more selective inclusion criteria for specific monogenic conditions as we aim to extend our research to a more diverse demographic. To facilitate this, we propose a clinical prioritization framework called 'SEPH,' which focuses on the following four key elements: severe outcome, early onset, prevalent incidence and high analytical performance (Extended Data Table 2). First, the condition under consideration should result in severe outcomes such as reduced lifespan, impaired mobility, intellectual disability, malformations, sensory impairment or immunodeficiency, with minimal phenotypic variability. This ensures that the identified conditions are most likely substantial and exhibit consistent characteristics, facilitating reliable predictions. Second, the onset of the condition should typically occur during infancy or childhood. Third, population prevalence data should be available that allows an accurate assessment of condition risk before and after the screening test. Conditions of higher prevalence should be prioritized to enhance cost-effectiveness. Finally, a screening test's high analytical sensitivity is crucial, ensuring that it can detect most pathogenic variants in the candidate gene. Following these criteria proposed in this study, priority was given to 37 genes (Extended Data Table 2). The rest of the genes are assigned low priority and can generally be excluded from screening in the broader population. Exceptions may be considered in special cases where invasive diagnostic procedures are declined, making prenatal cfDNA analysis the sole avenue for optimized perinatal management[49]. The SEPH framework highlights differing principles for condition selection in diverse patient populations that will guide our future population studies as a preliminary measure, using structured approaches in new screening method development[51,52]. The ultimate aim is to evolve into an evidence-based, quantifiable and objective methodology for the refinement of the abovementioned analytical elements. These include the appropriate quantification and categorization of condition severity for inclusion criteria, along with the formulation of robust, condition-specific guidelines for interpreting sequence variants in a prenatal setting. Achieving this objective requires extensive collaboration among clinical specialists, as demonstrated by ClinGen gene and condition curation studies, as well as the development of condition-specific sequence interpretation guidelines[53,54]. Beyond the analytical considerations, a comprehensive evaluation and resolution of various factors are imperative before implementing expanded prenatal cfDNA screening in the general obstetrical population. These encompass clinical utility validation, equitable test access, robust genetic counseling, informed public policy development, financial sustainability and the addressing of ethical and psychosocial implications[55–57]. Among these factors, genetic counseling is pivotal for the success of a prenatal screening program, as it aids expectant parents in making informed decisions about testing options and ensures accurate interpretation of test results. As demonstrated in the development of other new genetic tests, tackling the abovementioned complex issues before any clinical implementation will require rigorous studies and strong interdisciplinary collaboration[58].

In summary, this prospective, multicenter cohort study comparing cfDNA screening and diagnostic testing results supports the clinical validity of a comprehensive prenatal cfDNA screening including three of the most frequent causes of human genetic conditions—aneuploidies, microdeletions and monogenic variants. Given its reasonable accuracy and substantially improved detection rate, an expanded prenatal cfDNA screening merits consideration for further exploration as a tool for the noninvasive evaluation of fetal risks of heterogeneous genetic conditions.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-023-02774-x.

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Jinglan Zhang[1,2,3,4,12] ✉, Yanting Wu[1,5,12], Songchang Chen[1,12], Qiong Luo[2,6,12], Hui Xi[7,12], Jianli Li[4], Xiaomei Qin[4], Ying Peng[7], Na Ma[7], Bingxin Yang[8], Xiang Qiu[5], Weiliang Lu[5], Yuan Chen[2,6], Ying Jiang[2,6], Panpan Chen[2,6], Yifeng Liu[2,6], Chen Zhang[1], Zhiwei Zhang[4], Yu Xiong[5], Jie Shen[5], Huan Liang[5], Yunyun Ren[5], Chunmei Ying[5], Minyue Dong[2,6], Xiaotian Li[5], Congjian Xu[1,5], Hua Wang[7,9] ✉, Dan Zhang[2,6] ✉, Chenming Xu[1,5] ✉ & Hefeng Huang[1,2,5,8,10,11] ✉

[1]Obstetrics and Gynecology Hospital, Institute of Reproduction and Development, Fudan University, Shanghai, China. [2]Key Laboratory of Reproductive Genetics (Ministry of Education) and Department of Reproductive Endocrinology, Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China. [3]School of Medicine, Shanghai Jiao Tong University, Shanghai, China. [4]Beijing BioBiggen Technology Co., Ltd, Beijing, China. [5]Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China. [6]Key Laboratory of Women's Reproductive Health of Zhejiang Province, and Zhejiang Provincial Clinical Research Center for Child Health, Zhejiang University School of Medicine, Hangzhou, China. [7]National Health Commission (NHC) Key Laboratory of Birth Defect for Research and Prevention, Hunan Provincial Maternal and Child Health Care Hospital, Changsha, China. [8]International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China. [9]NHC Key Laboratory of Birth Defects Research, Prevention and Treatment, Hunan Provincial Maternal and Child Health Care Hospital, Changsha, China. [10]Research Units of Embryo Original Diseases, Chinese Academy of Medical Sciences, Shanghai, China. [11]Shanghai Frontiers Science Research Center of Reproduction and Development, Shanghai, China. [12]These authors contributed equally: Jinglan Zhang, Yanting Wu, Songchang Chen, Qiong Luo, Hui Xi. ✉e-mail: jinglanzhang@foxmail.com; wanghua213@aliyun.com; zhangdan@zju.edu.cn; chenming_xu2006@163.com; huanghefg@hotmail.com

Methods

Study design

This prospective cohort was an observational study designed to evaluate the clinical validity of a comprehensive prenatal cfDNA screening approach for a broad range of chromosomal and monogenic conditions by comparing the cfDNA and genetic diagnostic results. The screening panel included a total of seven frequent aneuploidies and nine microdeletion syndromes (Extended Data Table 1). In addition, dominant monogenic conditions associated with 75 genes were selected (Extended Data Table 2). To assess the performance metrics of the prenatal cfDNA screening test, cases were followed up to compare the screening results with the prenatal or postnatal diagnostic testing results. All participants were prospectively enrolled and followed up from 24 April 2021 to 10 September 2022 with the screening test being administered before diagnostic testing. This investigation was observational rather than interventional in nature, and the invasive diagnostic procedures were initiated solely based on clinical indications. In addition, the screening results were not reported unless they were consistent with those of diagnostic tests. All clinical pregnancy management decisions were based on the results of diagnostic testing, rather than the comprehensive cfDNA screening results, in accordance with current standard practice guidelines. None of the participants in the cohort had been involved in our previous studies.

This study has been reviewed and approved by the internal review board at the Obstetrics and Gynecology Hospital of Fudan University (2020-178). This clinical study led by the Obstetrics and Gynecology Hospital of Fudan University has received approval for the collection of human genetic resources in China from the Ministry of Science and Technology of China (2021-CJ0599). The trial registration number was ChiCTR2100045739 with a published study protocol[59].

Patient involvement and participant eligibility

Patients were involved in the conduct of this research during their visit seeking prenatal care. During the recruitment stage, the design, methods and outcome of the research and testing were informed by discussions with patients through a structured interview. Between 24 April 2021 and 10 September 2022, 1,191 pregnant women were consecutively enrolled and followed up from three tertiary hospitals in different provinces of China. Eligible women were ≥20 years old with a singleton pregnancy of ≥12 weeks gestation (in compliance with national regulation on the gestational age requirement for prenatal cfDNA screening in China) who did not undergo any prior prenatal diagnosis. Pregnancies with fetal structural anomalies (including nuchal translucency ≥3 mm), high-risk results from standard cfDNA screening or maternal serum screening\or previous pregnancy history suggestive of elevated risks for genetic conditions were assessed for enrollment. The enrollment for cases with isolated increased nuchal translucency was capped at 20% of the total with abnormal ultrasound findings. Exclusion criteria encompassed pregnant women with an age <20 years old, gestational age <12 weeks, one of the parents with or suspected to have a chromosomal abnormality, recent blood or organ transplantation, clinical history indicated for diagnostic testing of a known familial variant(s) in the panel and maternal malignancy during pregnancy. Genetic diagnosis was made by the analysis of samples collected from chorionic villus sampling, amniocentesis, products of conception or cord blood. Cases with no diagnostic testing results, failing assay quality control or enrolled not compliant with the inclusion criteria were also excluded. Written consent was received from all participants. Each participant provided consent for the publication of scientific findings, which may include genetic and clinical diagnoses, pregnancy outcomes and related demographic data such as age and gestational age.

The library preparation process for cfDNA sequencing

The comprehensive prenatal cfDNA screening used in this study was developed by Beijing BioBiggen Technology and involved a targeted sequencing method termed COATE-seq, as described previously[33]. A total of 10 ml of peripheral blood was collected from each participant, and plasma was separated by a standard two-step centrifugation process. A minimum of 1.8 ml of maternal plasma was first isolated from whole blood by centrifuging the collection tube at 1,600*g* for 15 min at a temperature of 4 °C. The plasma was then subjected to a second round of centrifugation at 16,000*g* for 10 min at 4 °C. The extraction of cfDNA was executed using the Magnetic Serum/Plasma Circulating DNA Maxi Kit (Tiangen).

The extracted cfDNA was first end-repaired following the manufacturer protocol (Nanodigbio), before being ligated at 20 °C for 15 min using adapters containing unique molecular indexes. A PCR was initiated to introduce the sample barcode, with the following parameters: initial denaturation at 98 °C for 2 min, followed by nine cycles of denaturation at 98 °C for 15 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. This was finalized by an extension step at 72 °C for 2 min. The resultant PCR products were then quantified using Qubit 1× dsDNA HS Assay Kits (Invitrogen).

For target enrichment, 12–36 samples were pooled and incubated at 65 °C for 16 h with hybridization probes per manufacturer protocol (Heristar). The DNA was then recovered, washed and purified with the Dynamag-270 magnetic beads (Invitrogen). Another PCR was performed to create the sequencing library, which involved an initial denaturation at 98 °C for 2 min, followed by 12 cycles of denaturation at 98 °C for 15 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s, before ending with a final extension at 72 °C for 2 min. Next, single-stranded circular DNA libraries were generated using the MGI-Easy Circularization Kit (MGI). The circular DNA was then converted into DNA nanoballs via rolling circle amplification, as per MGI's protocol. The concentration of the final sequencing library was measured using Qubit ssDNA Assay Kits (Invitrogen). The completed DNA library was finally sequenced on an MGISEQ-2000 sequencer from MGI, China, using a 2 × 100 paired-end mode.

The cfDNA analysis for single-gene variants, microdeletions and aneuploidies

The minimum threshold for sequencing depth was 200× for single-gene sequence variant calling. The mean coverage for the genes of interest across all samples was 1,387×, and more than 99.3% of the targeted regions on average in all samples met the minimum coverage requirement of 200×. The average gene-specific coverage meeting the minimum sequencing depth threshold (percentage of target regions with >200×) is provided in Extended Data Table 2. Mutect2 was used as the primary algorithm for variant calling, with the variant allele fraction threshold configured to a lower bound of zero (https://gatk.broadinstitute.org/hc/en-us/articles/360037593851-Mutect2). Two additional filtering methods were used in the identification of fetal monogenic variants following variant calling—allele-count distribution (ACD) and fetal–maternal insert-size distribution (FMID) as previously described[33]. For a specific variant under evaluation, it was considered more likely of fetal origin if its allele fraction (or alternative-allele count) fell within the expected range correlated with fetal fraction. If the log cumulative distribution function value for the β-binomial distribution ranged between −10 and −0.001, the variant passed the ACD filter. In the FMID filter, the insert size of each read containing an alternative allele was assessed to exclude reads with proximal insert sizes harboring the reference allele. Subsequently, the insert sizes of all remaining reads with either reference or alternative alleles were compared using the four statistical tests: Welch's *t* test, Kolmogorov–Smirnov test, Kruskal–Wallis *H* test and Mann–Whitney *U* test. In this phase, variants present on fragments with alternative alleles that exhibited statistically different lengths compared to those with reference alleles were retained (the minimum *P* value of the above four tests was ≤0.001). Next, to mitigate the risk of over-filtering variants, particularly in samples with low fetal fraction, a median insert-size

comparison was used to preserve variants present on shorter fragments where the median length of alternative-allele fragments was less than that of reference-allele fragments. Variants that failed to pass both the above ACD and FMID filters were marked as most likely of maternal origin or sequencing artifacts.

The test detects monogenic single-nucleotide variants and ≤3 bp insertions, deletions or indels in the coding exons and 10 bp into the intronic regions adjacent to the exon/intron junctions of targeted genes. It does not detect sequence variants in nontarget regions, exonic CNVs, dynamic variants, complex recombination or other structural variants. Variants located in regions complicated by high repetitive sequences, high GC content, homologous sequences or pseudogenes may not be detected. The test only reports pathogenic or likely pathogenic variants associated with severe outcomes, adhering to the ACMG guidelines[60], and excludes reporting benign, likely benign and variants of uncertain significance. The detection rate for each gene by the sequence analysis is listed in Extended Data Table 2. This test identifies target whole chromosome abnormalities but may not detect smaller aberrations within these chromosomes as described previously[33]. Similarly, the test detects target microdeletions covering the entire critical regions associated with the conditions and smaller deletions within these regions may not be detected.

The detection of maternal variants

The analysis includes a fragment length assessment, considering that maternal cfDNA typically presents longer fragment lengths in comparison to fetal cfDNA. When a variant is found on cfDNA fragments with lengths surpassing those of the reference allele fragments, an examination of maternal leukocytes is undertaken to investigate the possibility of maternal germline or mosaicism carrier status. Moreover, if the monogenic allelic fraction exceeds two s.d. above the expected fetal variant level, a maternal leukocyte test is also conducted. The s.d. for the fetal allelic fraction is calculated based on the single-nucleotide polymorphisms included in the chromosomal copy number analysis assay. The maternal test involves using cells collected from the buffy coat during plasma isolation. Regardless of the results from the above maternal test, a genetic diagnostic test for the fetus is always recommended.

Diagnostic testing for singe-gene conditions, microdeletions and aneuploidies

All participants in the final cohort (n = 1,090) who yielded either negative or positive cfDNA sequencing results had undergone at least one diagnostic test, using genomic DNA extracted from chorionic villi, amniocytes, cord blood or the product of conception. Different diagnostic tests were used as the reference methods for the targeted singe-gene conditions, microdeletions and aneuploidies in the prenatal cfDNA screening as described below.

NGS gene panel. This test uses a library construction kit (Nanodigmbio) and a targeted capture hybridization kit (IDT) for the preparation of DNA sequencing libraries. High-throughput sequencing is performed on the MGI-2000 (MGI) sequencers. All exonic regions and 10 bp intronic regions located both upstream and downstream of the exon–intron junctions of those 75 genes (the RefGene transcripts used in the Human Gene Mutation Database) included in the prenatal cfDNA screening test were sequenced. This test has an average sequencing depth of over 500× in targeted regions, with ≥99% of the target regions sequencing depth of over 20×. This test detects sequence variants including single-nucleotide variants and insertions, deletions or indels up to 20 nucleotides at an accuracy ≥99%. Dynamic variants, rearrangement variants and complex recombination variants are not detected. Reportable variants detected by NGS that are confounded by pseudogenes or homologous sequences detected in the NGS test are confirmed by locus-specific amplicon Sanger sequencing.

WES. This test employs the KAPA HyperExome (Roche) kit to capture and enrich DNA from the exon and neighboring splicing regions of the target genes. MGISEQ-2000 sequencing platform is used for sequencing. This test has an average sequencing depth of over 180× in targeted regions, with ≥95% of the target regions sequencing depth of over 20×. This test detects sequence variants including single-nucleotide variants and insertions, deletions or indels up to 20 nucleotides at an accuracy ≥99%, as well as exonic deletions at an accuracy ≥95%. This test only reports pathogenic, likely pathogenic, or variants of unknown clinical significance, not reporting likely benign or benign variants. Dynamic variants, rearrangement variants and complex recombination are not detected. This test may detect aneuploidies, absence of heterozygosity (AOH) ≥5 mb and certain dynamic variants with limited accuracy. This method cannot detect large fragment genomic CNVs (deletion/duplication interval <1 mb) and genomic structural variations (such as translocations, inversions, <5 mb AOH). This test does not detect all variants affected by highly repetitive low-complexity regions or pseudogenes.

Chromosomal microarray analysis. This test uses the Affymetrix CytoScan HD Array chip (Thermo Fisher Scientific), containing about 1.95 million CNV markers and approximately 750,000 SNP markers, for whole-genome chromosomal aneuploidies, microdeletions, microduplications and terminal deletions. This test can detect AOH. This test does not identify chromosomal balanced translocations, inversions, insertions or low percentage mosaicism. The test results are filtered using ChAS software and do not report duplications less than 500 kb, deletions less than 300 kb, polymorphic copy number changes indicated by public databases or AOH segments less than 10 mb.

Chromosome CNV-seq. The DNA is analyzed by NGS on an MGI (MGI) or Illumina platform (Illumina). This test detects aneuploidy of autosomes and sex chromosomes, deletions (≥1 mb), duplications (≥2 mb) and mosaicism (≥30%). This test does not detect uniparental disomy or AOH.

Karyotyping. The karyotype analysis involves the collection of cultured cells subjected to chromosomal preparation and G-banding (320 bands). The tests detect both numerical and structural changes of autosomes and sex chromosomes. This test may not detect microdeletions, duplications or abnormalities at the single-gene level.

Study outcomes

The outcomes of the study were the clinical validity of an expanded prenatal cfDNA screening and its detection rate for different types of genetic conditions causing fetal anomalies. Results for both screening and diagnostic testing performed on chorionic villus, amniocentesis, cord blood or products of conception were collected and compared for qualified participants. The clinical validity was measured by calculating the screening test sensitivity, specificity, PPV, NPV and the AUC. Only pregnant women who underwent diagnostic genetic testing were included in the study results, while those lacking any genetic diagnostic testing results were excluded. The detection rates of a diagnostic genetic variant associated with aneuploidies, microdeletions and monogenic conditions were measured for the entire cohort and with respect to different indications.

Postnatal follow-up

The study collected the postnatal follow-up data for the pregnancy outcomes of the participants by reviewing medical records, which included miscarriages, elective abortions, stillbirths and live-birth deliveries. When medical records of pregnancy outcomes were not available in the participating hospitals, participants were contacted by phone up to three attempts and up until 6 weeks after the expected delivery date. Pregnancy outcomes and clinical examination

results were evaluated to examine if they were consistent with the genetic diagnosis.

Data handling

Variant interpretation was carried out by at least one laboratory director certified by the American Board of Medical Genetics and Genomics. Only pathogenic and likely pathogenic variants associated with severe outcomes were reported, which were scored following well-established variant assessment criteria[60,61]. Positive variants were reported only when they were consistent with diagnostic testing. Experienced clinical geneticists provided post-test genetic counseling to participants regarding the interpretation of diagnostic results, the impact of these positive results and potential management options. Participants' prenatal screening and diagnostic test results, clinical examination findings and images and other relevant information were collected from the medical records and used for statistical analysis. Microsoft Excel was used for the clinical data collection.

Statistical analysis

The diagnostic testing results were compared to the prenatal cfDNA screening results to assess its clinical validity. Assay performance metrics were demonstrated by sensitivity, specificity, PPV and NPV, according to each category of abnormalities. Data were analyzed with respect to different indications. Before the start of this study, we performed a power analysis and planned to enroll at least 1,000 participants from whom we expected to detect at least 25 cases affected by the targeted chromosomal and monogenic conditions. This estimation was based on the detection rate among pregnancies with similar indications. The sample size in this study would allow a probability of 95% or above to observe a possible measuring error at the case level for both the chromosomal and monogenic conditions. Average ages were compared using a two-tailed *t* test for samples with unequal variances. For all calculations, *P* values less than 0.05 were considered statistically significant. The Clopper–Pearson method was used to calculate the test performance including sensitivity, specificity and positive and NPVs with exact 95% CIs. AUC was used to evaluate the prenatal cfDNA screening performance. The ROC curve was generated by computing sensitivity and specificity at each cutoff using Scikit-learn RocCurveDisplay (https://scikit-learn.org/).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The demographic data, clinical history, prenatal cfDNA screening, diagnostic test results and the diagnostic test methodologies of all 1,090 participants in the final cohort are within the paper and the Extended Data. All the pathogenic single-gene variants and the key phenotypes of the participants are available in the ClinVar database at https://www.ncbi.nlm.nih.gov/clinvar/submitters/508997/. The raw data files for all 1,090 participants are securely stored in an environment compliant with patients' privacy protection regulations within our laboratory and will be maintained for a minimum of ten years following publication. Access to these raw data files, unfiltered cfDNA gene sequencing data (VCF files) and locus-specific diagnostic sequencing results is available upon request from the corresponding author, J.Z. This process is to assure that patients' data privacy will be safeguarded and that the data will be used exclusively for noncommercial academic research purposes. All requests for data access must originate from an academic institution and be accompanied by verifiable affiliation (for example, a publicly accessible research investigator profile on the institution's website). Upon receipt of a qualified request, it will undergo review by a Data Privacy Committee (DPC), composed of two senior investigators from the study and an external reviewer, to verify that the data will be used exclusively for noncommercial, academic research purposes. After DPC approval, the execution of a Data Transfer Agreement is required, which will explicitly stipulate nondisclosure to third party and that the data are to be used solely for noncommercial, academic research activities. Qualified requests will be processed within a 3-week time frame. The hg38 reference genome sequence can be obtained at https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001405.40/.

Code availability

Customized computing code used in this study is available at https://github.com/Jinglan1/NIPS2/. Raw FASTQ were filtered and UMI-preprocessed using FASTP 0.21.0, https://github.com/OpenGene/fastp. The clean FASTQ files were aligned to hg38 human reference using BWA 0.7.17-r1188 (https://github.com/lh3/bwa) and then sorted by Samtools 1.9 (https://github.com/samtools/samtools/releases/). Consensus BAM files were generated by Gencore 0.15.0 and then finalized by BaseRecalibrator and ApplyBQSR GATK 4.1.8.0 followed by variant calling (https://gatk.broadinstitute.org). Raw variants were annotated by Annovar v2019-10-24 (https://annovar.openbioinformatics.org/).

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Author contributions

H.H., J.Z., C.-M.X., D.Z. and H.W. designed the study. H.H., J.Z., C.-M.X., D.Z., H.W., Y.W., S.C., Q.L., H.X., B.Y., C.Z., C.Y., C-J.X., J.L., J.S., M.D., N.M., P.C., W.L., X.Q., X.-M.Q., X.L., Y.L., Y.J., Y.P., Y.X., Y.C., Y.R. and Z.Z. conducted the clinical analyses. J.Z., J.L. and X-M.Q. conducted the statistical analyses. J.Z. wrote the paper. H.H., J.Z., D.Z., H.W. and C.-M.X. supervised the project.

Competing interests

J.Z., J.L., X.-M.Q. and Z.Z. are employees or shareholders of Beijing BioBiggen Technology or its subsidiaries and affiliates. A patent for the comprehensive noninvasive prenatal screening has been granted to the Beijing BioBiggen Technology (J.Z., J.L. and Z.Z.). The other authors declare no conflict of interest related to this work.

Additional information

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Correspondence and requests for materials should be addressed to Jinglan Zhang, Hua Wang, Dan Zhang, Chenming Xu or Hefeng Huang.

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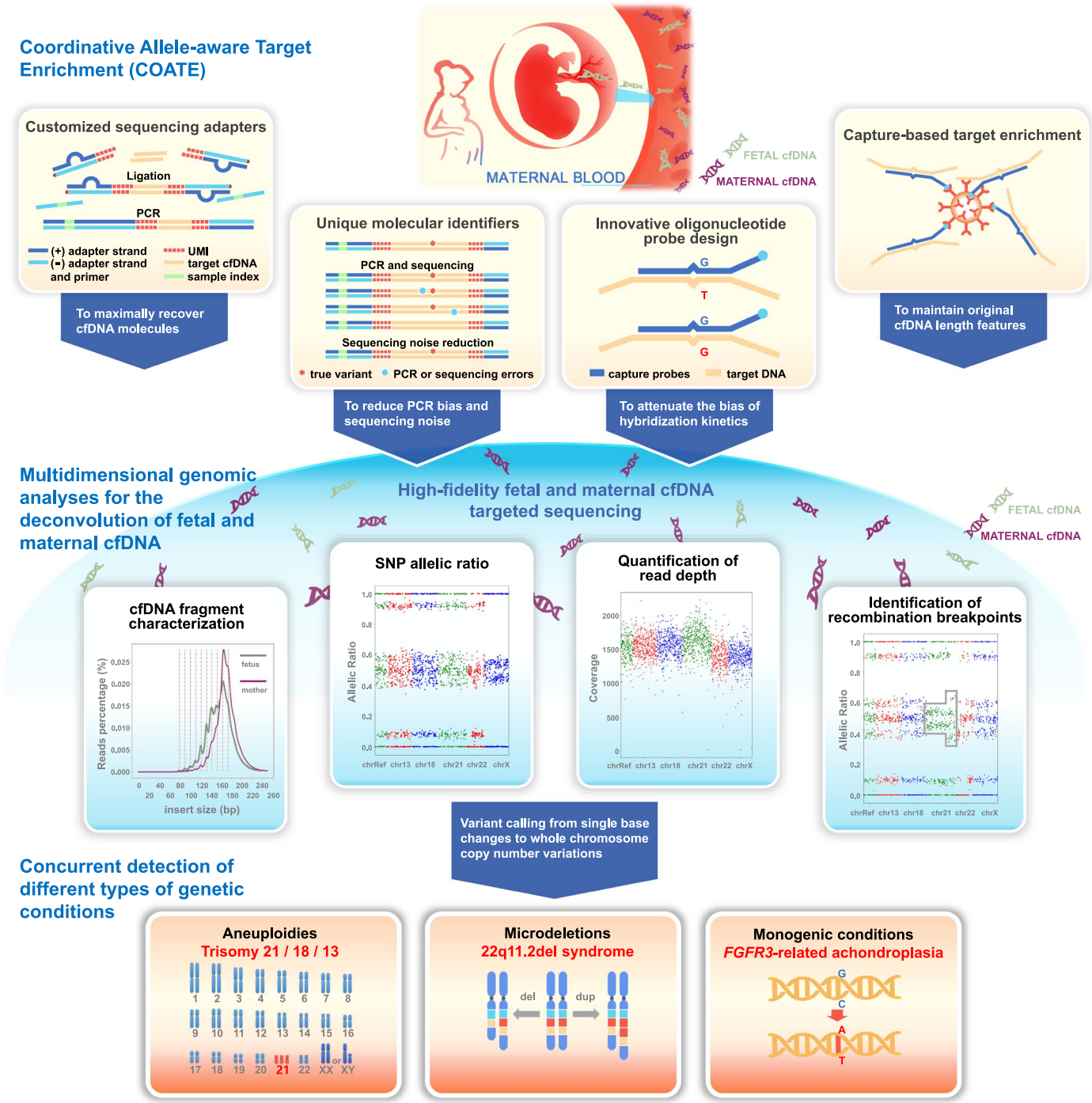

Extended Data Fig. 1 | The illustration of a comprehensive prenatal cell-free DNA screening test. The comprehensive prenatal cfDNA screening methodology utilizes a multi-faceted approach, involving new laboratory technologies, genomic algorithms and specialized condition interpretation analytics. Top panels: the test employs a tailored sequencing library construction process that combines customized adaptors for improved ligation efficiency, molecular indexing to curtail PCR-induced errors and capture-based hybridization to reduce allele drop-out, significantly increasing overall test accuracy for different types of genetic variants. Central to the method are the coordinative allele-aware target enrichment (COATE) probes which are designed to minimize the difference in hybridization equilibrium constants between reference and alternative alleles, which may not be perfectly complementary to either the wild-type or variant allele but reduce the enrichment bias introduced by conventional probes. Middle panels: fetus-specific genomic features, including cfDNA fragment length, meiotic error origin, meiotic recombination and recombination breakpoints, are used together to discern fetal monogenic and chromosomal variants. Bottom panels: condition-specific analytics are used for the interpretation of genetic variants following the American College of Medical Genetics guidelines on the analyses of sequence variants and chromosome copy-number variations. Only those classified as pathogenic or likely pathogenic variants following these guidelines are reported.

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Extended Data Table 1 | The targeted chromosomal conditions screened

Condition	Targeted region	Prevalence[1]
Trisomy 21	chr21	1:800
Trisomy 18	chr18	1:5,000
Trisomy 13	chr13	1:25,000
45X	chrX	1:2,000
47XXX	chrX	1:1,000
47XXY	chrX, chY	1:800
47XYY	chrX, chY	1:1,000
DiGeorge syndrome	chr22q11.2	1:4,000
1p36 deletion syndrome	chr1p36	1:5,000
2q33.1 deletion syndrome	chr2q33	NA
Angelman syndrome	chr15q11.2-q13	1:15,000
Prader-Willi syndrome	chr15q11.2-q13	1:22,500
Cri du Chat syndrome	chr5p15	1:30,000
Wolf-Hirschhorn syndrome	chr4p16	1:35000
Langer-Giedion syndrome	chr8q23q24	<1:1,000,000
Jacobsen syndrome	chr11q23q25	1:75,000

[1]Prevlance data were collected from the GeneReviews (https://www.ncbi.nlm.nih.gov/books/NBK1116/), Online Catalog of Human Genes and Genetic Disorders (https://omim.org/) and published literature when available. NA: not available.

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Extended Data Table 2 | The targeted monogenic conditions and prioritization assessment for screening

Gene	Condition	Sequencing detection rate[1]	Average gene coverage[2]	Detection rate of cfDNA screening	Condition prevalence[3]	Prioritization for general population screening study[4]
BRAF	Noonan spectrum disorder, cardiofaciocutaneous syndrome	>99%	99.2%	>98%		
CBL	Noonan spectrum disorder	92%	99.9%	92%		
HRAS	Noonan spectrum disorder, Costello syndrome	>99%	>99.9%	>99%		
KRAS	Noonan spectrum disorder, cardiofaciocutaneous syndrome	>99%	99.9%	>99%		
MAP2K1	Noonan spectrum disorder, cardiofaciocutaneous syndrome	>99%	>99.9%	>99%		
MAP2K2	Noonan spectrum disorder, cardiofaciocutaneous syndrome	>99%	99.5%	>99%		
NRAS	Noonan spectrum disorder	>99%	>99.9%	>99%	4-10:10,000	S, E, P, H
PTPN11	Noonan spectrum disorder, LEOPARD syndrome	>99%	99.5%	>99%		
RAF1	Noonan spectrum disorder	>99%	>99.9%	>99%		
RIT1	Noonan spectrum disorder	>99%	>99.9%	>99%		
SHOC2	Noonan spectrum disorder	67%	>99.9%	67%		
SOS1	Noonan spectrum disorder	>99%	99.9%	>99%		
SOS2	Noonan spectrum disorder	>99%	99.7%	>99%		
NIPBL	Cornelia de Lange syndrome	97%	99.7%	97%		
SMC1A	Cornelia de Lange syndrome	>99%	>99.9%	>99%		
SMC3	Cornelia de Lange syndrome	97%	99.9%	97%	1-10:100,000	S, E, P, H
RAD21	Cornelia de Lange syndrome	91%	>99.9%	91%		
HDAC8	Cornelia de Lange syndrome	90%	>99.9%	90%		
ALX4	parietal foramina	90%	>99.9%	90%	2-7:100,000	S, E, P, H
MSX2	parietal foramina	90%	>99.9%	90%		
COL1A1	osteogenesis imperfecta	95%	>99.9%	95%		
COL1A2	osteogenesis imperfecta	95%	99.8%	95%	5-7:100,000	S, E, P, H
IFITM5	osteogenesis imperfecta	>99%	>99.9%	>99%		
COL11A1	stickler syndrome, Marshall syndrome	99%	>99.9%	99%	1:10,000	S, E, P, H
COL2A1	stickler syndrome	99%	>99.9%	99%		
CDKL5	epileptic encephalopathy	79%	99.9%	79%	1:50,000	S, E, P, H
CHD7	CHARGE syndrome	98%	>99.9%	98%	6-12:100,000	S, E, P, H
EPHB4	lymphatic malformation	>99%	>99.9%	>99%	8-9:100,000	S, E, P, H
FGFR2	Apert syndrome	99%	>99.9%	99%	1-9:100,000	S, E, P, H
FGFR3	thanatophoric dysplasia, achondroplasia	99%	99.2%	98%	1-2:10,000	S, E, P, H
KMT2D	Kabuki syndrome	99%	99.3%	98%	2-3:100,000	S, E, P, H
MECP2	Rett syndrome	90%-95%	99.4%	90%-95%	4-10:100,000	S, E, P, H
NSD1	Sotos syndrome	45%-80%	97.1%	44%-78%	7-8:100,000	S, E, P, H
RUNX2	cleidocranial dysplasia, Metaphyseal dysplasia	80%	94.3%	76%	1-2:100,000	S, E, P, H
SOX9	campomelic dysplasia	90%-95%	97.3%	88%-92%	1-3:100,000	S, E, P, H
TSC1	tuberous sclerosis	94%	>99.9%	94%	1-2:10,000	S, E, P, H
TSC2	tuberous sclerosis	94%	>99.9%	94%		S, E, P, H
ASXL1	Bohring-Opitz syndrome	83%	98.7%	82%	<1:1,000,000	S, E, P*, H
CD96	C syndrome	>99%	>99.9%	>99%	1-9:1,000,000	S, E, P*, H
COL10A1	metaphyseal chondrodysplasia	>99%	>99.9%	>99%	3-6:1,000,000	S, E, P*, H
EBP	chondrodysplasia punctata	>99%	>99.9%	>99%	5-10:1,000,000	S, E, P*, H
FGFR1	trigonocephaly, Hartsfield syndrome	>99%	>99.9%	>99%	<1:1,000,000	S, E, P*, H
FLNB	Larsen syndrome	>99%	>99.9%	>99%	1-9:1,000,000	S, E, P*, H
GLI3	Pallister-Hall syndrome	95%	>99.9%	95%	<1:1,000,000	S, E, P*, H
HNRNPK	Au-Kline syndrome	94%	>99.9%	94%	<1:1,000,000	S, E, P*, H
KAT6B	genitopatellar syndrome, SBBYSS syndrome	98%	>99.9%	98%	<1:1,000,000	S, E, P*, H
NSDHL	CHILD syndrome, CK syndrome	88%	>99.9%	88%	<1:1,000,000	S, E, P*, H
RERE	neurodevelopmental disorder	95%	99.9%	95%	<1:1,000,000	S, E, P*, H
SKI	Shprintzen-Goldberg syndrome	>99%	95.7%	>95%	<1:1,000,000	S, E, P*, H
SLC25A24	fontaine progeroid syndrome	>99%	99.9%	>99%	<1:1,000,000	S, E, P*, H
SMAD4	Myhre syndrome	>99%	>99.9%	>99%	1:1,000,000	S, E, P*, H
SNRPB	cerebrocostomandibular syndrome	83%	>99.9%	83%	<1:1,000,000	S, E, P*, H
SPECC1L	Teebi hypertelorism syndrome 1	>99%	>99.9%	>99%	<1:1,000,000	S, E, P*, H
STAT3	autoimmune disease	99%	>99.9%	99%	<1:1,000,000	S, E, P*, H
TRAF7	cardiac, facial, and digital anomalies with developmental delay	>99%	>99.9%	>99%	<1:1,000,000	S, E, P*, H
FBN1	Marfan syndrome	90%-93%	99.8%	90%-93%	1-2:10,000	E, P, H
FREM1	trigonocephaly	90%	>99.9%	90%	1-5:10,000	E, P, H
LBR	Pelger-Huet anomaly	>99%	99.9%	>99%	1-2:10,000	E, P, H
LMNA	Hutchinson-Gilford progeria, cardiomyopathy	99%	>99.9%	99%	<1:1,000,000	E, P, H
NF1	neurofibromatosis	60%-90%	>99.9%	60%-90%	3-4:10,000	E, P, H
NF2	neurofibromatosis	75%	99.9%	75%		E, P, H
RYR1	congenital myopathy	>99%	98.7%	>98%	<1:1,000,000	E, P, H
TWIST1	craniosynostosis	72%	69.9%	50%	2-4:100,000	S, E, P
EFNB1	craniofrontonasal dysplasia	94%	>99.9%	94%	NA	S, E, H
ERF	craniosynostosis, Chitayat syndrome	94%	99.6%	94%	NA	S, E, H
PRKAR1A	carney complex	60%	>99.9%	60%	NA	S, E, H
PTH1R	metaphyseal chondrodysplasia	>99%	96.5%	>96%	NA	S, E, H
TGFBR1	Loeys-Dietz syndrome	>99%	93.1%	>92%	NA	S, E, H
TGFBR2	Loeys-Dietz syndrome	>99%	99.9%	>99%		S, E, H
TCF12	craniosynostosis	93%	>99.9%	93%	NA	S, E, H
ZIC1	structural brain anomalies and craniosynostosis	>99%	>99.9%	>99%	NA	S, E, H
PKD1	polycystic kidney disease	97%	undetermined	undetermined	1:1,000	S, P
PKD2	polycystic kidney disease	97%	94.8%			S, P
SMAD6	aortic valve disease, radioulnar synostosis	>99%	92.5%	>92%	NA	E, H
IHH	skeletal abnormality	81%	>99.9%	81%	NA	E, H

[1]Sequencing detection rate is the percentage of variants detectable by sequencing method among all pathogenic variants. The detection rate data were collected from GeneReviews (https://www.ncbi.nlm.nih.gov/books/NBK1116/) or calculated based on literature in the Human Gene Mutation Database database (https://my.qiagendigitalinsights.com/bbp/view/hgmd/pro/search_gene.php). [2] The average gene-specific coverage meeting the minimum sequencing depth threshold (percentage of target regions with >200×) was provided. [3] Prevalence data were cited from GeneReviews, Orphanet (https://www.orpha.net/consor/cgi-bin/index.php), Online Catalog of Human Genes and Genetic Disorders (https://omim.org/), MedlinePlus (https://medlineplus.gov/) and published literature when available. [4] Clinical prioritization criteria for conditions recommended in general population screening are based on public data and the findings of this study. S: conditions with severe outcomes (for example, shortened lifespan, impaired mobility, intellectual disability, malformation, sensory impairment, immunodeficiency, etc.) and no extreme phenotypic variability. E: conditions with early onset in infancy or childhood. P: conditions with known population prevalence. Conditions with a prevalence lower than 1:100,000 are marked with * assigned with a lower priority for general population. H: a high analytical performance in the screening test. NA: not available.

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Extended Data Table 3 | The detection rate of diagnostic genetic variants across different indications

Indication	Number of cases (%)	Chromosomal conditions (%)	Monogenic conditions (%)
Fetal structural anomalies	876 (80.4)	61 (7.0)	37 (4.2)
Lymphatic or effusion	46 (5.3)	15 (32.6)	2 (4.3)
Skeletal	85 (9.7)	1 (1.2)	20 (23.5)
Multisystem	73 (8.3)	14 (19.2)	3 (4.1)
Increased nuchal translucency	159 (18.2)	14 (8.8)	4 (2.5)
Cardiac	174 (19.9)	10 (5.7)	3 (1.7)
Brain	91 (10.4)	4 (4.4)	2 (2.2)
Craniofacial	35 (4.0)	2 (5.7)	0
Growth restriction	35 (4.0)	0	1 (2.9)
Renal	108 (12.3)	1 (0.9)	2 (1.9)
Spinal	12 (1.4)	0	0
Chest	17 (1.9)	0	0
Abdominal	41 (4.7)	0	0
Standard prenatal cfDNA screening high-risk results	86 (7.9)	35 (40.7)[1]	0
Maternal serum screening high-risk results	116 (10.6)	2 (1.7)	0
Clinical history suggestive of genetic conditions	12 (1.1)	0	0
Total	1,090	98 (9.0)	37 (3.4)

[1]All positive cases on standard prenatal cfDNA screening for chromosomal conditions but tested negative on the comprehensive prenatal cfDNA screening were confirmed negative by diagnostic testing.

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Extended Data Table 4 | Summary of fetuses affected by chromosomal conditions identified by comprehensive prenatal cfDNA screening and confirmed by diagnostic testing

Subject	GA (weeks)	MA (years)	Indication	FF (%)	Comprehensive prenatal cfDNA screening result	Diagnostic testing and pregnancy outcome
P38	21.1	38	NT 8.9 mm, bilateral clubfoot, ventricular septal defect	9.2	T21	amniocentesis; karyotype; elective abortion
P39	12.6	40	NT 4.3 mm	21.8	T21	amniocentesis; karyotype
P40	17.1	34	NT 4.8 mm	16.4	T21	amniocentesis; CMA and karyotype; elective abortion
P41	19.3	35	NT 4.2 mm	16.2	T21	amniocentesis; CMA and karyotype; elective abortion
P42	20.0	40	heart malformation	11.9	T21	product of conception; CNV-seq; elective abortion
P43	20.0	34	atrioventricular septal defect, pulmonary stenosis, nasal bone dysplasia	13.0	T21	product of conception; CNV-seq; elective abortion
P44	18.1	20	fetal choroidal cyst, nasal bone absent	17.1	T21	amniocentesis; CNV-seq, and CMA; elective abortion
P45	17.3	28	unclear nasal bone	10.0	T21	amniocentesis; CMA; elective abortion
P46	18.1	31	NT 3.9 mm	8.7	T21	amniocentesis; CMA
P47	17.0	34	NT 7.1 mm, nasal bone absent, ventricular septal defect	5.7	T21	amniocentesis; CNV-seq and CMA; elective abortion
P48	18.0	24	NT 3.7 mm	6.4	T21	amniocentesis; CNV-seq, CMA, and karyotype
P49	19.0	33	bright spots on the left ventricle	9.7	T21	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P50	18.0	32	NT 3.6 mm	11.6	T21	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P51	18.3	36	fetal hydrops, NT 5.8 mm	13.8	T21	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P52	18.3	38	bilateral choroidal cyst, nasal bone absent	11.4	T21	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P53	16.0	26	NF 6.0 mm, unclear nasal bone, cystic hygroma	10.8	T21	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P54	17.3	28	NF 6.9 mm, fetal edema	15.5	T21	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P55	17.0	38	NT 4.4 mm	9.5	T21	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P56	29.3	31	multiple malformations	17.9	T21	amniocentesis; CNV-seq; elective abortion
P57	17.0	35	NT 3.4 mm	23.9	T21	amniocentesis; CNV-seq; elective abortion
P58	31.0	37	right ventricular hypertrophy, right ventricular wall thickening, pericardial effusion	11.5	T21	amniocentesis; CNV-seq
P59	35.0	33	short femur and humerus	13.6	T21	amniocentesis; CNV-seq
P60	16.0	31	NT 3.8 mm, cystic hygroma, fetal hydrops	12.5	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P61	17.1	30	cystic hygroma	13.4	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P62	17.9	33	NT 3.4 mm, reversed a-wave in ductus venosus	7.1	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P63	18.3	42	bilateral choroidal cyst	8.1	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P64	17.6	35	nasal bone dysplasia, bilateral lateral ventriculomegaly	8.6	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P65	17.7	32	NT 3.6 mm	10.7	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P66	13.1	27	NT 4.0 mm, cystic hygroma	11.1	T18	amniocentesis; CMA and karyotype; elective abortion
P67	12.0	24	cranial malformations, omphalocele, radial dysplasia	3.1	T18	product of conception; CNV-seq; elective abortion
P68	21.6	28	open spina bifida, cardiac defects	8.4	T18	product of conception; CNV-seq; elective abortion
P69	18.4	39	fetal hydrops	10.3	T18	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P70	14.4	25	fetal hydrops, increased NT	8.0	T18	amniocentesis; CNV-seq; elective abortion
P71	13.8	25	NT 5.1, radial longitudinal deficiency, cardiac defects	7.5	T18	amniocentesis; CNV-seq; elective abortion

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Extended Data Table 4 (continued) | Summary of fetuses affected by chromosomal conditions identified by comprehensive prenatal cfDNA screening and confirmed by diagnostic testing

Subject	GA (weeks)	MA (years)	Indication	FF (%)	Comprehensive prenatal cfDNA screening result	Diagnostic testing and pregnancy outcome
P72	19.8	38	NT 4.7 mm, multiple malformations	15.1	T18	amniocentesis; CNV-seq, and karyotype; elective abortion
P73	16.4	30	NT 3.4 mm, reversed a-wave in ductus venosus, ventricular septal defect	8.7	T18	amniocentesis; CNV-seq, and karyotype; elective abortion
P74	17.1	32	NT 3.5 mm	8.8	T18	amniocentesis; CNV-seq, and karyotype; elective abortion
P75	12.7	37	cardiac defects	10.7	T18	amniocentesis; WES, CNV-seq, and karyotype; elective abortion
P76	21.4	31	multiple malformations	7.4	T13	product of conception; CNV-seq; elective abortion
P77	13.0	26	multiple malformations, fetal hydrops, brain and heart abnormalities, increased NT	9.4	T13	amniocentesis; CNV-seq, and CMA; elective abortion
P78	17.0	28	NT 6.9 mm	8.0	T13	amniocentesis; CNV-seq, and karyotype; elective abortion
P79	14.0	31	forebrain malformation	4.4	T13	amniocentesis; WES; elective abortion
P80	12.1	29	cystic hygroma	8.6	45X	amniocentesis; CNV-seq
P81	12.7	28	fetal hydrops, cystic hygroma	7.0	45X	amniocentesis; CNV-seq and karyotype; elective abortion
P82	15.1	33	fetal hydrops	5.3	45X	chorionic villus; CNV-seq and karyotype; elective abortion
P83	15.3	36	bilateral pleural effusion, fetal hydrops, cystic hygroma	8.2	45X	product of conception; CNV-seq; elective abortion
P84	13.0	26	subcutaneous soft tissue thickening, cystic hygroma	11.6	45X	amniocentesis; CNV-seq, and CMA; elective abortion
P85	13.9	40	cystic hygroma	15.1	45X	amniocentesis; CNV-seq, and CMA; elective abortion
P86	14.7	29	bilateral pleural effusion, fetal hydrops, NT 7.2mm	7.5	45X	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P87	18.0	34	left temporal cyst, bilateral choroidal cyst	7.1	45X	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P88	13.0	24	NT 8.2 mm, cystic hygroma	5.2	45X	amniocentesis; CNV-seq, CMA, and karyotype;
P89	24.6	29	NT 7.9 mm, reversed a-wave in ductus venosus, bilateral pleural effusion	6.9	45X	amniocentesis; CNV-seq, and karyotype; elective abortion
P90	17.7	28	cystic hygroma	11.7	45X	amniocentesis; CNV-seq, and karyotype; elective abortion
P91	33.3	27	spinal abnormalities, short femur	32.2	45X	amniocentesis; CNV-seq, and CMA; elective abortion
P92	18.7	32	NT 5.4 mm	12.1	47XYY	amniocentesis; CMA and karyotype
P93	22.4	26	cardiac defects	8.9	22q11.2del	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P94	25.0	27	double outlet right ventricle, ventricular septal defect	16.4	22q11.2del	amniocentesis; CNV-seq
P95	25.0	29	ventricular septal defect, bilateral renal pelvis separation	7.3	22q11.2del	amniocentesis; CNV-seq, and karyotype; elective abortion
P96	26.3	37	suspected tetralogy of Fallot	12.4	22q11.2del	amniocentesis; CNV-seq, and karyotype; elective abortion
P97	17.4	30	multiple malformations	7.8	4p16del	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion
P98	16.0	30	NT3.8 mm	6.4	4p16del	amniocentesis; CNV-seq, and karyotype; elective abortion
P99	19.7	42	standard prenatal cfDNA screening high risk	16.9	T21	amniocentesis; CNV-seq; elective abortion
P100	18.6	35	standard prenatal cfDNA screening high risk	9.2	T21	amniocentesis; CNV-seq; elective abortion
P101	18.0	27	standard prenatal cfDNA screening high risk	11.1	T21	amniocentesis; CNV-seq, and CMA; elective abortion
P102	18.9	37	standard prenatal cfDNA screening high risk	8.0	T21	amniocentesis; CNV-seq, and CMA; elective abortion
P103	18.3	41	standard prenatal cfDNA screening high risk	11.4	T21	amniocentesis; CNV-seq, CMA, and karyotype; elective abortion

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Extended Data Table 4 (continued) | Summary of fetuses affected by chromosomal conditions identified by comprehensive prenatal cfDNA screening and confirmed by diagnostic testing

Subject	GA (weeks)	MA (years)	Indication	FF (%)	Comprehensive prenatal cfDNA screening result	Diagnostic testing and pregnancy outcome
P104	22.0	36	standard prenatal cfDNA screening high risk	14.8	T21	amniocentesis; CNV-seq; elective abortion
P105	19.0	40	standard prenatal cfDNA screening high risk	4.7	T21	amniocentesis; CNV-seq; elective abortion
P106	17.3	39	standard prenatal cfDNA screening high risk	17.0	T21	amniocentesis; CNV-seq
P107	19.1	33	standard prenatal cfDNA screening high risk	18.2	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P108	19.3	21	standard prenatal cfDNA screening high risk	12.3	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P109	18.0	36	standard prenatal cfDNA screening high risk	6.7	T21	amniocentesis; CNV-seq, and karyotype
P110	16.4	36	standard prenatal cfDNA screening high risk	11.9	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P111	19.6	43	standard prenatal cfDNA screening high risk	8.8	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P112	23.8	23	standard prenatal cfDNA screening high risk	14.1	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P113	17.4	31	standard prenatal cfDNA screening high risk	7.8	T21	amniocentesis; CNV-seq, and karyotype; elective abortion
P114	18.6	44	standard prenatal cfDNA screening high risk	11.2	T18	amniocentesis; CNV-seq, and karyotype; elective abortion
P115	20.6	28	standard prenatal cfDNA screening high risk	8.6	T18	amniocentesis; CNV-seq, and karyotype; elective abortion
P116	18.3	35	standard prenatal cfDNA screening high risk	16.0	T13	amniocentesis; CNV-seq, and karyotype; elective abortion
P117	17.6	30	standard prenatal cfDNA screening high risk	3.3	45X	amniocentesis; CNV-seq, and karyotype; elective abortion
P118	22.4	28	standard prenatal cfDNA screening high risk	8.2	45X	amniocentesis; CNV-seq
P119	19.0	29	standard prenatal cfDNA screening high risk	10.5	45X	amniocentesis; CNV-seq; elective abortion
P120	17.4	29	standard prenatal cfDNA screening high risk	7.2	47XXX	amniocentesis; CNV-seq, and karyotype; elective abortion
P121	19.1	20	standard prenatal cfDNA screening high risk	8.2	47XXX	amniocentesis; CNV-seq; liveborn
P122	17.7	29	standard prenatal cfDNA screening high risk	8.9	47XXX	amniocentesis; CNV-seq, and karyotype; elective abortion
P123	19.9	40	standard prenatal cfDNA screening high risk	13.5	47XXX	amniocentesis; CNV-seq, and karyotype; liveborn
P124	17.4	31	standard prenatal cfDNA screening high risk	7.7	47XXX	amniocentesis; CNV-seq, and karyotype; liveborn
P125	17.3	28	standard prenatal cfDNA screening high risk	12.6	47XXX	amniocentesis; CNV-seq, and karyotype; liveborn
P126	19.6	32	standard prenatal cfDNA screening high risk	12.0	47XXY	amniocentesis; CNV-seq, and karyotype; elective abortion
P127	18.6	38	standard prenatal cfDNA screening high risk	14.1	47XXY	amniocentesis; CNV-seq, and karyotype; elective abortion
P128	18.6	38	standard prenatal cfDNA screening high risk	13.9	47XYY	amniocentesis; CNV-seq; liveborn
P129	17.9	29	standard prenatal cfDNA screening high risk	9.1	47XYY	amniocentesis; CNV-seq, and karyotype; liveborn
P130	18.0	29	standard prenatal cfDNA screening high risk	7.3	47XYY	amniocentesis; CNV-seq, and karyotype
P131	16.1	41	standard prenatal cfDNA screening high risk	11.4	22q11.2del	amniocentesis; CNV-seq
P132	17.0	31	standard prenatal cfDNA screening high risk	10.5	22q11.2del	amniocentesis; CNV-seq, and karyotype; elective abortion
P133	18.4	27	standard prenatal cfDNA screening high risk	16.8	4p16del	amniocentesis; CNV-seq; elective abortion
P134	18.9	27	maternal serum screening high risk	17.6	T21	amniocentesis; CNV-seq, and karyotype
P135	17.0	40	maternal serum screening high risk	5.0	47XYY	amniocentesis; CNV-seq, and karyotype; liveborn

GA: gestational age. MA: maternal age. FF: fetal fraction. CNV-seq: next-generation sequencing based chromosomal copy-number variation analysis. CMA: chromosomal microarray analysis. NT: nuchal translucency. NF: Nuchal fold.

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Extended Data Table 5 | Cases with false screening results

Subject	GA (weeks)	MA (years)	Indication	FF (%)	Standard prenatal cfDNA screening	Comprehensive prenatal cfDNA screening	Diagnostic testing results and pregnancy outcome
F1	23.0	32	standard prenatal cfDNA screening high risk	12.3	T13	T13	amniocentesis; negative on CNV-seq, CMA, and karyotype
F2	16.0	39	standard prenatal cfDNA screening high risk	12.6	T13	T13	amniocentesis; negative on CNV-seq
F3	22.6	26	standard prenatal cfDNA screening high risk	12.3	T13	T13	amniocentesis; negative on CNV-seq, and karyotype
F4	16.6	33	standard prenatal cfDNA screening high risk	8.6	T13	T13	amniocentesis; negative on CNV-seq, and karyotype; liveborn
F5	17.0	30	maternal serum screening and standard prenatal cfDNA screening high risk	12.8	45X	45X	amniocentesis; negative on CNV-seq, CMA, and karyotype; liveborn
F6	27.6	29	standard prenatal cfDNA screening high risk	10.7	45X	45X	amniocentesis; negative on CNV-seq, CMA, and karyotype; liveborn
F7	17.1	32	clinical history suggestive of genetic conditions and increased nuchal translucency	10.3	Low risk	Low risk	amniocentesis; T21 on CNV-seq and karyotype; spontaneous abortion
F8	27.0	28	cardiac defects	17.9	Low risk	Low risk	product of conception; T21 on CNV-seq; elective abortion

GA: gestation age. MA: maternal age. FF: fetal fraction. CNV-seq: next-generation sequencing based chromosomal copy-number variation analysis. CMA: chromosomal microarray analysis. T21: trisomy 21.

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Extended Data Table 6 | The diagnostic testing results and pregnancy outcomes of pregnancies with negative prenatal cfDNA screening results

Subject	Gestation age (wks)	Maternal age (yrs)	Indication	FF (%)	Comprehensive prenatal cfDNA screening result	Diagnostic testing					Specimen type	Pregnancy outcome
						CMA	Karyotyping	CNV-seq	NGS-SGD	WES		
N1	22.6	30	Multicystic kidney dysplasia	16.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N2	17.0	29	NT 3.6 mm	11.5	Low risk	-	Normal	-	-	-	Amniocytes	-
N3	22.6	40	Bilateral renal pelvis separation, bright spots on the ventricle	7.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N4	23.1	32	NF 6.4 mm, dilated left renal pelvis, short femur	8.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N5	22.9	29	Suspected hand polydactyly, abnormality of the 2nd toe	11.0	Low risk	-	Normal	-	-	-	Product of conception	-
N6	17.7	25	NT 3.8 mm	11.7	Low risk	-	Normal	-	-	-	Amniocytes	-
N7	27.0	26	Pulmonary stenosis	16.0	Low risk	-	Normal	-	-	-	Product of conception	-
N8	22.1	33	Right multicystic kidney dysplasia	7.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N9	13.6	31	Right multicystic kidney dysplasia	11.8	Low risk	Normal	-	-	-	-	Amniocytes	Elective abortion
N10	25.7	24	Pulmonary stenosis	12.1	Low risk	Normal	-	-	-	-	Product of conception	Liveborn
N11	17.4	35	NT 4.1 mm	10.2	Low risk	Normal	Normal	-	-	-	Amniocytes	Liveborn
N12	25.1	32	Cardiac defects, bilateral mild hydronephrosis	6.7	Low risk	Normal	Normal	-	-	-	Product of conception	Elective abortion
N13	18.1	24	NT 4.9 mm, tricuspid regurgitation	10.9	Low risk	Normal	Normal	-	-	-	Amniocytes	Elective abortion
N14	26.1	32	Cardiac defects	22.5	Low risk	Normal	Normal	-	-	-	Product of conception	Elective abortion
N15	24.0	32	Right ear malformation	12.3	Low risk	Normal	Normal	-	-	-	Product of conception	Elective abortion
N16	21.1	26	Multiple abnormalities, bilateral lateral ventriculomegaly	14.1	Low risk	Normal	Normal	-	-	-	Amniocytes	Elective abortion
N17	18.0	35	Cystic hygroma	7.4	Low risk	Normal	Normal	-	-	-	Amniocytes	Liveborn
N18	28.0	31	Cardiac defects	16.8	Low risk	Normal	Normal	-	-	-	Product of conception	Liveborn
N19	17.0	28	Left choroidal cyst	17.1	Low risk	Normal	Normal	-	-	-	Amniocytes	Liveborn
N20	22.0	29	Bilateral choroidal cyst	13.6	Low risk	Normal	Normal	-	-	-	Product of conception	Elective abortion
N21	29.0	31	Brain dysplasia	18.6	Low risk	Normal	Normal	-	-	-	Product of conception	Elective abortion
N22	18.0	39	NT 3.7 mm	11.4	Low risk	Normal	Normal	-	-	-	Amniocytes	Liveborn
N23	19.6	27	Venous catheter absent	8.1	Low risk	Normal	Normal	-	-	-	Amniocytes	-
N24	25.0	32	Bilateral talipes valgus, big toe abnormality	12.7	Low risk	Normal	Normal	Normal	-	-	Amniocytes	Liveborn
N25	30.0	30	Cardiac and bipedal sonograms changed	16.5	Low risk	-	-	Normal	-	-	Product of conception	Elective abortion
N26	16.0	31	NT 4.7 mm	16.5	Low risk	-	-	Normal	-	-	Amniocytes	Liveborn
N27	22.6	37	NF 6.1 mm, left kidney dysplasia, spinal abnormalities	14.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N28	25.3	30	Hydronephrosis, posterior urethral valve	11.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N29	24.0	32	Spina bifida, meningocele	3.5	Low risk	-	-	Normal	-	-	Product of conception	-
N30	26.0	29	Small left heart, coarctation of the ascending aortic arch	14.4	Low risk	-	-	Normal	-	-	Product of conception	Elective abortion
N31	23.9	34	Suspected clubfoot	9.5	Low risk	-	-	Normal	-	-	Amniocytes	Liveborn
N32	22.4	28	Ventricular septal defect	16.1	Low risk	-	-	Normal	-	-	Product of conception	Elective abortion
N33	24.6	30	Bilatera pleural effusion, fetal hydrops	7.1	Low risk	-	-	Normal	-	-	Product of conception	-
N34	17.9	28	Increased NT, osteogenesis dysplasia	8.7	Low risk	-	Normal	Normal	-	-	Amniocytes	Elective abortion
N35	26.6	32	Renal agenesis	15.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N36	29.1	26	Dilated bilateral renal pelvis	16.1	Low risk	Normal	-	Normal	Normal	-	Amniocytes	-
N37	24.9	30	Echogenic bowel, nasal bone absent	6.7	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N38	22.9	33	Short nasal bone	8.9	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N39	26.3	26	Ectopic kidney	11.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N40	25.7	26	Bilateral lateral ventriculomegaly, unclear nasal bone	15.9	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N41	20.6	24	Right clubfoot, bilateral choroidal cyst	5.9	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N42	24.3	33	Cardiac defects, decreased middle cerebral artery pulsatility index	6.6	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N43	24.4	38	Cardiovascular system abnormality, stomach bubble absent	14.1	Low risk	-	Normal	Normal	-	-	Product of conception	Elective abortion
N44	13.7	30	Cystic hygroma	8.8	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N45	32.6	29	Right hydronephrosis	20.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N46	23.6	34	Complete transposition of the great arteries	9.7	Low risk	-	Normal	Normal	-	-	Product of conception	Elective abortion
N47	32.0	26	Duplicated kidney, dilated of the ureter	25.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N48	24.9	32	Ventricular septal defect, renal sinus separation	9.9	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N49	24.3	28	Ventricular septal defect	12.3	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N50	12.9	26	NT 3.2 mm	8.6	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N51	23.3	32	NF 7.6 mm	8.6	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N52	28.4	29	Small fetus, bowel enlarged	14.7	Low risk	-	Normal	Normal	-	-	Product of conception	Elective abortion
N53	27.3	26	Bilateral lateral ventriculomegaly, hydrocephalus	14.5	Low risk	-	Normal	Normal	-	-	Product of conception	Elective abortion
N54	22.4	35	Left lateral ventriculomegaly	14.5	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N55	25.9	30	Left kidney absent	10.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N56	23.1	36	NF 6.5 mm	6.3	Low risk	-	Normal	Normal	-	-	Amniocytes	Elective abortion
N57	12.6	30	NT 6.8 mm	10.1	Low risk	-	Normal	Normal	-	-	Amniocytes	-
N58	13.4	30	NT 3.8 mm	11.9	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N59	13.4	38	NT 5.4 mm	10.1	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N60	12.7	25	Cystic hygroma	8.7	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N61	23.0	27	Left choroidal cyst	10.0	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N62	29.3	31	Multiple abnormalities	17.8	Low risk	-	Normal	Normal	-	-	Product of conception	-
N63	18.0	31	Ventricular septal defect	14.8	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N64	24.4	33	Right lateral ventriculomegaly, coronary sinus enlargement	10.3	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N65	24.6	31	Clubfoot	16.5	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N66	24.7	26	Cardiovascular system abnormality	11.6	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N67	26.4	31	Left duplicated kidney, ureter abnormalities	14.9	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N68	18.0	35	Bright spots on the left ventricle	8.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N69	16.4	29	NT 3.2 mm	7.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N70	24.7	26	Bright spots on the left ventricle, vagus right subclavian artery	10.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N71	14.7	29	Anencephaly	15.3	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N72	23.7	26	Clubfoot, bilateral finger overlap, short mandible, scalp edema	5.5	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N73	23.0	31	Complex cardiac dysplasia, left cardiac dysplasia	7.6	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N74	13.6	30	Omphalocele, cardiac defects, tetralogy of Fallot	11.0	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N75	27.3	20	Dilated bilateral renal pelvis, polyhydramnios	21.1	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N76	22.6	37	NF 6.8 mm	10.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N77	27.4	31	NF 6.8 mm	14.7	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N78	21.3	24	Spina abnormalities with Arnold-Chiari malformation	9.2	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N79	17.3	33	NT 3.6 mm	10.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N80	23.3	33	Dilated left renal pelvis	6.6	Low risk	-	Normal	Normal	-	-	Product of conception	Elective abortion
N81	22.6	35	Lumbar hemivertebrae	11.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N82	23.0	34	Dilated bilateral renal pelvis	16.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N83	31.0	32	Short long bones	20.5	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N84	12.3	25	Cystic hygroma, fetal hydrops, nasal bone absent	6.4	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N85	14.0	23	Skull aura absent	8.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N86	26.4	34	Right ventricle dysplasia, tricuspid stenosis	10.6	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N87	24.4	36	Left renal agenesis	13.6	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N88	15.9	22	Hydrocephalus	21.6	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N89	13.7	23	Multiple abnormalities	14.5	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N90	27.0	30	Brain neoplasm	18.4	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N91	31.9	26	Hydrocephalus	30.0	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N92	23.6	28	Cleft lip and palate	8.1	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N93	25.1	33	Hydronephrosis, finger and toe malformations	13.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N94	20.1	28	Bilateral choroidal cyst, bright spots on the left ventricle	12.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N95	25.3	32	Cardiac defects, overriding aorta, pulmonic stenosis	13.7	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N96	24.0	29	Left heart hypoplasia, tricuspid valve abnormalities	13.2	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N97	17.0	20	Skull absent, multiple abnormalities	7.2	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N98	32.7	27	Bright spots on the right liver	24.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N99	22.4	37	Cystic hygroma	8.3	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N100	25.0	30	Spinal abnormalities	8.1	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N101	30.9	25	Cardiac defects, fetal hydrops	27.9	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N102	13.1	28	Multiple malformations	12.8	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N103	12.7	33	Fetal hydrops	8.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N104	19.3	29	Curved bilateral femur	16.4	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N105	21.3	45	Cardiac defects, skeletal dysplasia, spinal abnormalities	7.3	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N106	23.4	40	Multiple abnormalities, dysgenesis of the corpus callosum	17.4	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N107	19.1	26	Ventricular septal defect, left radius absent, single umbilical artery	8.5	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N108	30.6	30	Short long bones	15.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N109	30.1	26	Growth restriction, abnormal skull morphology	19.4	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N110	23.0	26	Left dilated renal pelvis, dilated renal calices	12.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N111	23.4	26	Congenital cardiopathy	13.0	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N112	19.9	25	Spinal abnormalities, cerebellar dysplasia, clubfoot	7.6	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N113	22.1	24	Kidney dysplasia, ureterocele, bright spots on the left ventricle	8.6	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N114	25.0	28	Multiple malformations	11.7	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N115	23.7	24	Left hand abnormalities	7.9	Low risk	-	-	Normal	Normal	-	Product of conception	-
N116	27.7	32	Growth restriction	6.5	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N117	30.3	31	Echogenic bowel	8.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N118	28.0	27	Kidney dysplasia	6.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N119	29.4	30	Multiple abnormalities	14.5	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N120	29.1	33	Ventricular septal defect	19.7	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N121	31.0	25	Bilateral lateral ventriculomegaly	24.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N122	12.7	35	Limbs abnormalities	10.3	Low risk	-	-	Normal	Normal	-	Product of conception	-

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Extended Data Table 6 (continued) | The diagnostic testing results and pregnancy outcomes of pregnancies with negative prenatal cfDNA screening results

Subject	Gestation age (wks)	Maternal age (yrs)	Indication	FF (%)	Comprehensive prenatal cfDNA screening result	Diagnostic testing					Specimen type	Pregnancy outcome
						CMA	Karyotyping	CNV-seq	NGS-SGD	WES		
N123	34.3	27	Small fetus	21.0	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N124	26.4	28	Small cerebellum, left kidney dysplasia	15.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N125	33.0	39	Left choroidal cyst	27.6	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N126	27.0	35	Growth restriction, posterior fossa cyst, cerebellar vermis hypoplasia	15.4	Low risk	-	-	Normal	Normal	-	Product of conception	-
N127	30.0	32	Ventricular septal defect, polyhydramnios	16.6	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N128	33.3	27	Decreased head circumference	23.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N129	22.0	30	Dilated bilateral renal pelvis, single umbilical artery	11.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N130	25.1	33	Ventricular septal defect	12.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N131	24.0	35	Spinal abnormalities	11.3	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N132	30.6	28	Right hydronephrosis	15.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N133	31.7	39	Small fetus	20.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N134	29.9	36	Small fetus	27.7	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N135	30.9	27	Ventricular septal defect	27.4	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N136	21.4	29	Left hydronephrosis	7.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N137	30.0	25	Intracranial morphology abnormalities	28.1	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N138	25.7	32	Bilateral lateral ventriculomegaly	10.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N139	16.1	33	NT 4.6 mm	10.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N140	32.4	28	Suspected arachnoid cyst	33.7	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N141	30.0	25	Small fetus	10.6	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N142	23.4	34	NF 6.2 mm, dilated bilateral renal pelvis, atrial septal aneurysm	15.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N143	30.4	30	Polyhydramnios	21.1	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N144	32.3	27	Cystic hygroma	14.4	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N145	23.9	41	Right pelvict kidney	11.4	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N146	29.0	21	Short long bones	21.9	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N147	17.4	30	NT 4.0 mm	8.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N148	27.9	30	NT 3.6 mm	14.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N149	14.1	36	Gastroschisis and visceral ectropion	9.4	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N150	30.0	34	Right pleural effusion	17.8	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N151	27.9	28	Single umbilical artery	17.3	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N152	22.6	31	Aberrant right subclavian artery	6.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N153	17.7	29	Multiple abnormalities	13.1	Low risk	-	Normal	Normal	Normal	-	Product of conception	Elective abortion
N154	27.6	32	External genitalia abnormalities	23.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N155	27.6	28	Hyperechogenic kidneys, increased of cardiothoracic area ratio	14.5	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	-
N156	25.6	35	Left lower leg abnormalities	19.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N157	26.3	25	Ventricular septal defect	15.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N158	30.4	31	Microcephaly	24.6	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N159	19.6	20	Fetal hydrops, left pleural effusion, ectopia cordis	11.2	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N160	21.3	26	Micrognathia, ventricular septal defect	18.5	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N161	17.4	28	NT 4.0 mm	10.4	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N162	14.0	28	Right ventricle dysplasia, pulmonary artery atresia or severe stenosis	14.5	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N163	22.4	30	NF 6.2 mm, dilated right renal pelvis	14.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N164	16.7	29	NT 5.8 mm	11.1	Low risk	Normal	-	Normal	Normal	-	Amniocytes	-
N165	20.7	28	Renal agenesis, hand polydactyly	8.1	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N166	18.0	35	NT 3.9 mm	5.7	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N167	17.9	27	NT 4.2 mm, unclear nasal bone	8.1	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N168	24.6	31	Short femur, lateral ventriculomegaly, single umbilical artery	10.2	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N169	23.6	29	Right renal agenesis, single umbilical artery	9.2	Low risk		Normal	Normal	Normal	-	Amniocytes	Liveborn
N170	13.9	32	Acrania	16.3	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N171	27.3	39	Gastrointestinal atresia	17.4	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N172	24.0	25	Complete transposition of the great arteries	9.1	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N173	14.0	28	Lower limb malformation, vertebral bowing	9.0	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N174	25.7	21	Bilateral lateral ventriculomegaly, facial structure abnormalities	17.1	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N175	13.3	28	Cystic hygroma	9.1	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N176	31.6	31	Occipital neoplasm	22.6	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N177	25.0	24	Lumbosacral tumor	14.3	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N178	13.0	24	Multiple abnormalities	7.2	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N179	18.3	28	Choroidal cyst, situs inversus stomach bubble, left inferior vena cava	7.3	Low risk	Normal	-	Normal	Normal	-	Amniocytes	-
N180	14.7	36	Cardiac defects, single atrium and single ventricle	4.0	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N181	25.1	27	Ventricular septal defect	19.4	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N182	13.7	33	NT 4.0 mm, cystic hygroma	11.8	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N183	19.7	36	Right choroidal cyst	5.1	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N184	15.4	38	Single left ventricle, coarctation of aorta	10.0	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N185	24.9	28	Intracranial cystic lesion	25.8	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N186	25.0	29	Bilateral clubfoot	25.5	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N187	25.1	33	Pulmonic stenosis	7.3	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N188	31.0	29	Pulmonary stenosis	21.5	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Liveborn
N189	17.9	31	NT 3.2 mm	5.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N190	18.7	29	NT 3.5 mm	2.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N191	20.1	35	NT 3.2 mm	12.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N192	18.3	34	NT 3.3 mm	8.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N193	17.3	27	NT 3.2 mm	20.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N194	16.0	28	NT 3.2 mm	7.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N195	17.0	24	NT 4.5 mm, cystic hygroma	9.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N196	25.9	27	Unclear nasal bone, aberrant right subclavian artery	10.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N197	22.6	31	Dilated left renal pelvis, bright spots on the left ventricle	12.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N198	20.6	29	NT 11.6 mm, unclear nasal bone	8.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N199	26.7	28	Cardiac defects	14.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N200	19.1	32	NT 4.2 mm	22.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N201	16.0	32	NT 7.4 mm, bilateral clubfoot	9.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N202	26.0	26	Cardiac defects, gallbladder absent, bilateral renal pelvis separation	7.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N203	17.0	29	NT 3.6 mm, right choroidal cyst	4.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N204	23.3	37	Dilated left renal pelvis	10.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N205	17.4	33	NT 5.9 mm	7.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N206	17.7	34	NF 7.1 mm, NT 4.2 mm	7.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N207	23.3	25	Digestive system abnormalities	16.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N208	24.1	26	Hand polydactyly	11.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N209	23.3	32	Bilateral renal pelvis separation	13.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N210	26.0	27	Situs inversus viscerum	18.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N211	31.6	32	Growth restriction	18.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N212	26.4	29	Bilateral renal pelvis separation, bright spots on the left ventricle	19.7	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N213	19.0	28	Hydrocephalus, cerebellar dysplasia	18.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N214	17.0	21	NT 4.0 mm	15.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N215	24.9	27	Cardiac defects	14.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N216	20.1	31	Echogenic bowel	8.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N217	17.0	33	Aortic arch	8.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N218	16.1	30	NT 5.3 mm	4.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N219	25.1	32	Left kidney dysplasia	7.0	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N220	16.0	24	NT 3.3 mm	15.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N221	28.6	31	Dilated bilateral renal pelvis	10.9	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N222	24.6	25	Nasal bone absent	12.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N223	18.6	31	NT 3.4 mm	8.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N224	24.1	28	Bilateral clubfoot, left hand hanging	4.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N225	17.6	35	NT 3.2 mm	9.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N226	24.0	34	Oligohydramnios, increased placental thickness	13.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N227	18.0	34	NT 4.1 mm	7.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N228	18.6	29	Unclear nasal bone	15.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N229	24.0	31	Bilateral renal pelvis separation	19.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N230	17.1	31	NT 3.8 mm	20.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N231	21.4	35	Cleft lip and palate, ventricular septal defect	16.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N232	21.1	35	Hyperechogenic kidneys	16.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N233	18.3	34	NT 3.8 mm	9.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N234	18.7	32	NT 3.0 mm, bilateral choroidal cyst	14.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N235	16.0	27	NT 4.1 mm	6.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N236	20.0	32	NT 3.2 mm, ductus venosus absent	5.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N237	25.0	28	Pulmonary stenosis, tricuspid regurgitation	6.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N238	23.4	31	Unilateral choroidal cyst, bilateral lateral ventriculomegaly	8.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N239	21.0	27	Bright spots on the left ventricle	9.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N240	20.0	24	Cystic hygroma	4.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N241	24.7	37	Double bubble syndrome, duodenal atresia	12.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N242	18.1	26	NT 3.8 mm	5.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N243	25.0	29	Micrognathia	20.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N244	18.0	26	NT 3.0 mm, bilateral choroidal cyst	7.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn

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Extended Data Table 6 (continued) | The diagnostic testing results and pregnancy outcomes of pregnancies with negative prenatal cfDNA screening results

Subject	Gestation age (wks)	Maternal age (yrs)	Indication	FF (%)	Comprehensive prenatal cfDNA screening result	Diagnostic testing					Specimen type	Pregnancy outcome
						CMA	Karyotyping	CNV-seq	NGS-SGD	WES		
N245	23.7	29	Dilated bilateral renal pelvis	10.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N246	29.0	25	Bilateral pyelectasis, echogenic bowel	11.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N247	25.1	30	Tetralogy of Fallot	19.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N248	16.9	32	Cystic hygroma	15.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N249	23.9	38	Cleft lip and palate	7.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N250	18.4	31	NT 5.0 mm	11.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N251	25.0	29	Cleft lip and palate	12.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N252	19.0	29	NT 4.4 mm	9.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N253	19.7	30	Lateral ventriculomegaly, left renal pelvis separation, choroidal cys	9.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N254	25.9	38	Cardiac defects	12.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N255	25.3	33	Complete transposition of the great arteries, ventricular septal defect	8.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N256	25.9	37	Fetal growth restriction	12.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N257	28.6	30	Multiple abnormalities	18.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N258	24.0	30	Multicystic kidney dysplasia, pinna malformation	8.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N259	27.0	33	Ventricular septal defect, ringlike pancreas, souble bubble sign	13.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N260	28.0	26	Cardiac defects	12.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N261	17.7	31	NT 4.3 mm	17.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N262	25.0	27	Bilateral lateral ventriculomegaly	10.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N263	25.4	24	Hyperechogenic left hemidiaphragm, bright spots on the left ventricle	12.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N264	25.0	34	Gastrointestinal tract abnormalities	10.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N265	19.6	24	Ventricular septal defect	7.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N266	18.4	35	NT 4.3 mm, bilateral choroidal cys, bright spots on the left ventricle	5.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N267	18.1	32	Cardiac defects	10.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N268	25.0	32	Tetralogy of Fallot	10.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N269	17.0	32	NT 5.6 mm, unclear nasal bone	7.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N270	27.4	34	Fetal growth restriction	10.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N271	26.4	38	Duodenal obstruction	13.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N272	31.0	25	Bilateral lateral ventriculomegaly	19.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N273	28.0	33	Ear malformation	16.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N274	26.1	31	Left ear malformation	8.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N275	25.0	31	Right hydronephrosis, urethral obstruction	17.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N276	26.6	32	Left ear malformation	10.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N277	19.6	37	Bilateral renal agenesis	8.3	Low risk	Normal	-	-	-	-	Amniocytes	Elective abortion
N278	25.4	30	Pelvic ectopic kidney, multicystic kidney dysplasia	16.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N279	28.0	27	Bilateral clubfoot	21.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N280	19.3	34	Hydronephrosis	11.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N281	22.3	29	Bilateral hydronephrosis	16.1	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N282	18.4	38	Bilateral renal pelvis separation, choroidal cyst	9.0	Low risk	Normal	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N283	26.7	36	Suspected tetralogy of Fallot	12.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N284	25.9	28	Double bubble sign, duodenal atresia	14.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N285	26.9	29	Cardiac defects, single umbilical artery, tetralogy of Fallot	10.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N286	30.3	34	Right kidney dysplasia, reduced renal corticomedullary differentiation	25.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N287	25.9	25	Left ectopic kidney, multicystic kidney dysplasia	11.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N288	20.0	30	Unclear nasal bone	11.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N289	24.7	36	Ventricular septal defect, coarctation of aorta	9.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N290	19.3	28	Cystic hygroma	7.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N291	33.1	28	Left multicystic kidney dysplasia	21.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N292	28.0	34	Abnormal inferior vena cava	23.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N293	26.0	31	Tethered cord, spina bifida	11.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N294	29.9	25	Short femur, lateral ventriculomegaly	20.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N295	27.3	33	Situs inversus viscerum, ventricular septal defect, holoprosencephaly	10.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N296	26.7	27	Cardiac defects, tetralogy of Fallot	31.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N297	25.7	35	Ventricular septal defect	7.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N298	17.6	28	Bilateral lateral ventriculomegaly	16.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N299	27.6	30	Dilated bowel	14.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N300	25.0	35	Single atrium and single ventricle, pulmonic stenosis	12.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N301	28.3	36	Left renal cyst	16.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N302	18.1	29	NT 5.6 mm	11.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N303	27.9	31	Echogenic bowel, enlarged posterior fossa	16.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N304	26.9	27	Right lateral ventriculomegaly, bilateral choroidal cys	19.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N305	19.1	30	NT 11.0 mm, coarctation of aorta, ventricular septal defect	19.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N306	20.0	32	Abnormal liver sonography, bilateral choroidal cys, fetal malformation	8.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N307	24.7	34	Enlarged bilateral renal, multiple sinusoid	11.2	Low risk	-	Normal	-	-	-	Product of conception	Liveborn
N308	30.0	30	Gastrointestinal tract malformation	22.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N309	27.4	37	Cardiac defects, tetralogy of Fallot	26.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N310	25.1	31	Right clubfoot	10.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N311	19.3	26	NT 6.3 mm, rocker bottom foot	12.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N312	23.4	25	Bilateral clubfoot	8.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N313	20.3	34	Bilateral choroidal cys, bright spots on the ventricle	15.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N314	23.0	35	Diaphragmatic hernia	7.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N315	31.7	26	Growth restriction	17.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N316	26.1	31	Left diaphragmatic hernia	13.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N317	25.7	37	Double bubble sign, gastrointestinal obstruction	14.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N318	19.0	31	NT 3.9 mm, echogenic bowel	10.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N319	18.3	30	Cystic hygroma, left choroidal cyst	7.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N320	23.1	27	Pulmonary cystadenoma	11.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N321	25.7	30	Bilateral lateral ventriculomegaly	11.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N322	30.0	39	Growth restriction	20.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N323	25.6	28	Ventricular septal defect, portal vein dysgenesis	12.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N324	25.3	27	Vascular tumor	13.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N325	23.7	28	Gastrointestinal tract malformation	13.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N326	25.0	34	Right pulmonary cystadenoma	10.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N327	25.6	30	Ventricular septal defect, single umbilical artery	12.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N328	18.9	28	Ectopic kidney, polycystic kidney dysplasia	6.6	Low risk	-	-	Normal	Normal	-	Product of conception	-
N329	29.9	26	Echogenic bowel	19.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N330	23.7	29	Dilated bilateral renal pelvis	16.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N331	25.0	26	Unclear nasal bone	13.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N332	26.9	37	Tetralogy of Fallot	7.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N333	23.3	28	Vascular tumor	17.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N334	24.0	24	Cardiac defects, left inferior vena cava, tetralogy of Fallot	11.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N335	25.0	23	Unclear nasal bone	5.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N336	26.9	27	Cardiac defects, ventricular septal defect, coarctation of aorta	14.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N337	20.0	37	Bilateral choroidal cys	8.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N338	30.1	32	Bilateral lateral ventriculomegaly	21.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N339	23.0	26	Ventricular septal defect	11.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N340	25.4	27	Double bubble sign, polyhydramnios	14.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N341	23.7	32	Bilateral choroidal cys	10.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N342	32.0	25	Bilateral enlarged kidney	26.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N343	24.3	35	Bright spots on the left ventricle, renal pelvis separation,	9.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N344	23.0	29	Nasal bone absent	10.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N345	28.6	25	Bilateral lateral ventriculomegaly, persistent left superior vena cava	19.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N346	26.7	34	Short femur, hypospadias, cardiac defects	11.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N347	19.0	23	NT 3.8 mm	12.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N348	18.9	26	NT 3.6 mm	9.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N349	19.3	34	Bilateral choroidal cys	8.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N350	24.4	25	Bilateral lateral ventriculomegaly, hydrocephalus	9.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N351	26.0	31	Dilated bilateral renal pelvis, aberrant right subclavian artery	6.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N352	17.1	27	NT 4.5 mm	7.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N353	18.7	26	NT 5.8 mm	12.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N354	19.4	29	Fetal hydrops	5.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N355	31.0	33	Enlarged bilateral renal, double inferior vena cava	34.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N356	29.6	36	Enlarged bilateral renal, polyhydramnios	26.7	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N357	18.4	29	Craniocerebral and vertebral abnormalities	9.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N358	21.4	35	Bilateral choroidal cys	12.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N359	30.6	27	Short long bones, small fetus	15.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N360	31.6	26	Bilateral lateral ventriculomegaly, echogenic bowel	9.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N361	26.0	23	Ventricular septal defect	11.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N362	18.7	27	Increased NT	10.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N363	26.0	31	Pulmonary veins abnormalities	7.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N364	19.0	28	Increased NT, cystic hygroma	13.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N365	17.0	26	NT 5.0 mm, cystic hygroma	6.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N366	17.4	30	NT 4.2 mm	8.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-

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Subject	Gestation age (wks)	Maternal age (yrs)	Indication	FF (%)	Comprehensive prenatal cfDNA screening result	Diagnostic testing					Specimen type	Pregnancy outcome
						CMA	Karyotyping	CNV-seq	NGS-SGD	WES		
N367	25.1	38	Ventricular septal defect	22.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N368	26.0	27	Short limbs	13.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N369	31.3	24	Hypoplastic colon, bright spots on the left ventricle	14.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N370	26.4	28	Left clubfoot	16.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N371	27.4	29	Enlarged bilateral renal, right hydronephrosis	25.0	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N372	13.0	30	Cystic hygroma	21.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N373	27.1	26	Echogenic bowel	21.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N374	17.3	30	NT 5.9 mm	6.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N375	18.1	25	NT 4.3 mm	3.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N376	19.1	27	NT 3.8 mm	8.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N377	25.4	28	External genitalia abnormalities	6.9	Low risk	-	Normal	-	-	-	Product of conception	-
N378	26.0	34	Cardiac defects	13.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N379	26.3	30	Growth restriction	17.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N380	18.1	32	Bilateral choroidal cys, large bladder	9.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N381	23.0	39	Ventricular septal defect, right pulmonary cystadenoma	30.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N382	17.0	34	Bilateral choroidal cys	7.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N383	20.0	30	Right choroidal cys	12.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N384	18.6	34	Choroidal cys	18.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N385	18.3	34	Left choroidal cyst	7.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N386	24.3	31	Bilateral choroidal cys	11.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N387	19.1	26	Bilateral choroidal cys	11.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N388	18.0	41	Bilateral choroidal cys	3.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N389	19.0	31	Right choroidal cyst	11.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N390	28.0	26	Brain cyst	9.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N391	25.1	34	Aplasia/hypoplasia of the corpus callosum	8.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N392	26.0	35	Fetal hydrops, pleural effusion	35.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N393	24.3	33	Low liver echo, strong intestinal echo, mild tricuspid regurgitation	17.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N394	18.3	22	NT 3.4 mm, ventricular septal defect	6.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N395	18.6	30	NT 3.4 mm	12.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N396	20.7	27	Spots on the left ventricle, bilateral renal pelvis separation	9.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N397	17.0	32	Increased echogenicity of the umbilical cord root	13.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N398	28.0	22	Meconium peritonitis	8.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N399	29.7	22	Fetal hydrops, pleural and celiac effusion	30.1	Low risk	Normal	Normal	Normal	Normal	-	Product of conception	Elective abortion
N400	25.0	25	Hyperechogenic left chest cavity	17.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N401	26.4	40	Gallbladder absent	13.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N402	16.3	28	Megacystis, single umbilical artery	9.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N403	24.0	32	Single umbilical artery	14.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N404	21.4	33	Venous catheter absent	14.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N405	25.4	32	Interrupted inferior vena cava	7.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N406	25.1	34	Aortic arch with mirror image branching	15.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N407	30.0	24	Right aortic arch, aberrant left subclavian artery	30.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N408	25.6	25	Pulmonic stenosis	18.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N409	32.4	37	Hepatomegaly	23.3	Low risk	-	-	Normal	Normal	-	Amniocytes	
N410	30.7	27	External genitalia abnormalities	20.7	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N411	21.0	34	Systemic skeletal dysplasia, short limbs, narrow chest cavity	7.3	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N412	23.1	32	Abnormality of prenatal development	12.0	Low risk	-	-	Normal	Normal	-	Amniocytes	
N413	24.0	22	Short long bones	24.6	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N414	25.0	27	Cardiovascular system abnormalities	15.0	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N415	24.0	28	Cystic hygroma	19.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N416	22.0	32	Right renal agenesis, single umbilical artery, double inferior vena cava	13.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N417	32.3	34	Short long bones	30.1	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N418	30.0	28	Short long bones	21.3	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N419	19.0	32	Short long bones	9.1	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N420	20.1	33	Posterior cranial fossa and cardiac sonographic changed	17.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N421	15.7	31	Increased NT, fetal hydrops, omphalocele, ectopia cordis	7.6	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N422	27.0	20	Suspected tetralogy of Fallot	19.4	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N423	26.3	29	Right kidney dysplasia, cardiovascular system abnormality	9.7	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N424	28.1	20	Short long bones	13.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N425	30.6	25	Bilateral lateral ventriculomegaly	37.3	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N426	30.0	25	Bilateral lateral ventriculomegaly	19.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N427	27.4	33	Small cerebellum, bilateral lateral ventriculomegaly	13.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N428	29.9	27	Spinal abnormalities	11.6	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N429	28.1	40	Multiple malformations	13.3	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N430	27.6	36	Bilateral lateral ventriculomegaly	22.1	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N431	28.7	24	Bilateral lateral ventriculomegaly	25.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N432	31.1	29	Ventricular septal defect	22.9	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N433	33.3	33	Small fetus, suspected nasolacrimal duct cyst,	14.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N434	29.9	32	Echogenic bowel	24.2	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N435	34.0	26	Cardiac defects	32.3	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N436	31.3	31	Small fetus	27.9	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N437	32.0	31	Short long bones	16.2	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N438	33.6	30	Dilatation of the right ureter, hydronephrosis	17.0	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N439	34.9	28	Small fetus	31.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N440	31.6	34	Abnormal inferior vena cava course	13.4	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N441	29.4	36	Ventricular septal defect	19.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N442	20.9	33	Multiple abnormalities	16.3	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N443	24.1	31	Ventricular septal defect	11.3	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N444	31.0	31	Left lateral ventriculomegaly	33.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N445	30.6	30	Right umbilical vein	16.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N446	31.9	33	Double right renal artery, aberrant right subclavian artery	27.3	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N447	25.1	30	Bilateral lateral ventriculomegaly	16.0	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N448	30.0	35	Left lateral ventriculomegaly	29.1	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N449	17.6	27	NT 4.0 mm	6.8	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N450	30.0	33	Ventricular septal defect	25.6	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N451	25.0	35	Cardiac defects (left coronary artery atrophy into right ventricle)	12.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N452	18.6	30	Nasal bone hypoplasia	9.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N453	19.0	27	Right kidney dysplasia, ureter abnormalities, polyhydramnios	28.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N454	31.7	29	Small fetus	29.0	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N455	31.0	29	Omphalocele, bilateral choroidal cyst	26.0	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N456	29.6	33	Ventricular septal defect	16.6	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N457	27.4	33	Right kidney dysplasia, polyhydramnios	28.8	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N458	27.9	31	Pelvic cyst	31.7	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N459	32.0	33	Ventricular septal defect	26.3	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N460	21.0	39	Cyst under the tongue	16.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N461	29.7	22	Cystic hygroma	23.0	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N462	30.0	26	Ventricular septal defect, arrhythmia, tricuspid regurgitation	34.3	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N463	18.4	33	Clubfoot	6.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N464	30.1	35	Intracranial morphology abnormalities	10.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N465	33.0	23	Small fetus	25.5	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N466	29.0	23	Renal dysplasia, nasal bone hypoplasia	20.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N467	29.3	24	Ventricular septal defect	29.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N468	31.9	26	Small fetus	10.9	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N469	21.1	27	NT 4.0 mm	7.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N470	33.4	30	Ventricular septal defect	21.2	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N471	28.3	29	Nasal bone hypoplasia	8.3	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N472	27.0	38	Bright spots on the heart	20.0	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N473	27.6	22	Small fetus	14.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N474	24.3	40	Spinal abnormalities	12.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N475	35.0	35	Small fetus	21.5	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N476	28.9	29	Low-set right kidney	15.8	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N477	27.4	23	Ventricular septal defect	22.0	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N478	26.3	37	Cardiovascular system abnormality	18.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N479	31.4	33	Small fetus	20.6	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N480	30.6	31	Enlarged posterior fossa, pericardial effusion	18.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N481	31.0	29	Celiac effusion	20.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N482	30.0	26	Vascular abnormalities	19.8	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N483	23.6	25	Aberrant right subclavian artery	7.3	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N484	17.9	30	NT 3.3 mm	8.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N485	16.7	37	NT 3.4 mm	8.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N486	16.4	32	NT 3.1 mm	9.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N487	22.7	31	Nasal bone absent	11.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N488	25.1	36	Short femur	11.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn

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Extended Data Table 6 (continued) | The diagnostic testing results and pregnancy outcomes of pregnancies with negative prenatal cfDNA screening results

Subject	Gestation age (wks)	Maternal age (yrs)	Indication	FF (%)	Comprehensive prenatal cfDNA screening result	Diagnostic testing					Specimen type	Pregnancy outcome
						CMA	Karyotyping	CNV-seq	NGS-SGD	WES		
N489	24.1	30	Right aortic arch, aberrant left subclavian artery	12.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N490	29.4	31	Cardiovascular system abnormality	18.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N491	23.1	33	Ventricular septal defect	8.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N492	23.6	26	Bright spots on the left ventricle, echogenic bowel	16.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N493	24.7	30	Headform abnormality	12.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N494	25.6	29	Right kidney dysplasia	8.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N495	27.0	29	Enlarged bilateral renal, bilateral lateral ventriculomegaly	16.7	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N496	24.3	22	Right aortic arc, aberrant left subclavian artery	21.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N497	22.0	28	Dilated bilateral renal pelvis, hydronephrosis, ventricular septal defect	8.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N498	23.9	30	Small fetus, single umbilical artery	9.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N499	29.4	28	NT 3.3 mm, bilateral lateral ventriculomegaly	27.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N500	24.1	37	Cardiovascular system abnormalities, pulmonic stenosis	14.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N501	25.0	23	Dilated left renal pelvis, hydronephrosis, multicystic kidney dysplasia	13.5	Low risk	-	-	Normal	-	-	Product of conception	Elective abortion
N502	27.1	27	Right clubfoot, left lateral ventriculomegaly	29.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N503	28.9	29	Dilated left renal pelvis, hydronephrosis, nasolacrimal duct cyst	24.8	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N504	24.6	31	Decreased biparietal diameter, short nasal bone	19.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N505	22.4	32	NF 6.3 mm, decreased head circumference	10.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N506	27.1	33	Porta hepatis cystic, dilated common bile duct, single umbilical artery	9.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N507	18.4	36	Cystic hygroma	9.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N508	24.7	36	Ventricular septal defect, abnormal ear morphology	17.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N509	25.4	33	Short femur, decreased biparietal diameter and head circumference	14.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N510	24.6	32	Short femur and humerus	14.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N511	24.3	31	Small feuts, echogenic bowe, right clubfoot	5.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N512	17.1	30	Cystic hygroma	6.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N513	24.7	33	Left kidney dysplasia	26.5	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N514	21.4	36	Short femur, decreased head circumference, bilateral choroidal cyst	9.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N515	22.1	34	Nasal bone absent	11.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N516	16.7	27	Unclear nasal bone	12.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N517	27.7	36	Small fetus,echogenic bowel,	23.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N518	24.6	29	Ventricular septal defect, bilateral choroidal cyst	7.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N519	23.0	32	NF 9.7 mm	12.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N520	25.0	28	Small fetus, decreased head and abdominal circumference	7.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N521	23.1	27	Bilateral lateral ventriculomegaly, polyhydramnios	7.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N522	22.1	31	NF 7.0 mm, short nasal bone	9.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N523	21.6	32	Left chest hypoplasia	10.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N524	26.1	30	Short femur and humerus	9.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N525	23.0	36	Echogenic bowel, bright spots on the left ventricle	9.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N526	24.4	29	Cardiovascular system abnormalities, echogenic bowel	10.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N527	24.0	28	Butterfly vertebrae	11.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N528	25.3	39	Left kidney dysplasia, cardiovascular system abnormality	20.7	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N529	23.0	25	Cardiovascular system abnormality	9.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N530	16.9	29	NT 4.5 mm	5.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N531	23.7	30	Left kidney dysplasia, multicystic kidney dysplasia	21.9	Low risk	-	Normal	Normal	-	-	Product of conception	Liveborn
N532	29.9	28	Short femur and humerus	31.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N533	25.1	27	Ventricular septal defect	11.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N534	16.3	27	NT 4.8 mm	8.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N535	22.9	27	NF 6.8 mm	8.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N536	24.0	27	Left kidney dysplasia, polycystic kidney dysplasia	21.6	Low risk	-	-	Normal	-	-	Product of conception	Elective abortion
N537	22.4	33	Cardiovascular system abnormality	13.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N538	22.3	40	NF 6.5 mm	9.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N539	22.6	37	NF 6.9 mm	16.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N540	23.3	29	NF 6.4 mm	9.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N541	32.3	29	Cardiac defects	21.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N542	24.1	24	Left kidney dysplasia	14.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N543	24.6	26	NF 6.9 mm	9.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N544	23.0	30	NF 8.5 mm	15.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N545	25.7	29	Right lateral ventriculomegaly	10.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N546	23.4	28	NF 6.6 mm	9.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N547	23.4	30	NF 6.7 mm	16.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N548	17.1	23	Increased NT	4.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N549	22.3	35	NF 7.8 mm	16.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N550	16.1	28	NT 4.8 mm	7.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N551	16.4	33	Cystic hygroma	5.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N552	23.6	24	Microcephaly	12.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N553	24.9	26	Situs inversus viscerum, tricuspid regurgitation	9.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N554	30.4	27	Abnormal morphology and position of stomach bubble, dilated bowel	12.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N555	25.0	26	Thickened soft tissue, limbs abnormalities	19.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N556	22.1	28	NF 6.4 mm	10.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N557	30.4	26	NF 6.6 mm	11.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N558	30.4	35	Small fetus	14.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N559	23.6	30	Tetralogy of Fallot	14.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N560	22.7	27	NF 6.9 mm	19.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N561	22.9	30	Duodenal atresia, cystic lesion below the liver	13.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N562	24.7	23	Echogenic bowel, nasal bone hypoplasia	11.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N563	17.6	29	Cystic hygroma	10.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N564	22.0	32	Short femur, decreased head circumference	17.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N565	25.9	35	Left kidney dysplasia, polycystic kidney dysplasias	24.3	Low risk	-	Normal	-	-	-	Product of conception	Elective abortion
N566	19.9	29	Left kidney dysplasia, polycystic kidney dysplasias	8.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N567	25.1	25	Decreased biparietal diameter and head circumference	16.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N568	25.1	27	Ventricular septal defect, persistent left superior vena cava	12.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N569	16.4	26	NT 3.7 mm	6.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N570	28.1	26	Short femur, dilated bilateral renal pelvis	10.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N571	23.9	25	Dilated bilateral renal pelvis, parenchymal echo above the diaphragm	6.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N572	24.4	30	Increased NT, short femur and humerus, cardiac defects	17.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N573	16.3	31	NT 5.6 mm	8.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N574	25.7	30	Persistent left superior cavity aortic arch with narrow isthmus	21.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N575	23.0	33	Bilateral lateral ventriculomegaly	8.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N576	22.6	35	Cardiac defects, tetralogy of Fallot	17.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N577	28.4	25	NT 4.5 mm, ductus venosus absent	11.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N578	24.7	32	Short femur and humerus, decreased head circumference	7.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N579	26.6	38	Echogenic bowel, dilated renal pelvis	14.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N580	24.3	27	Renal pelvis separation, single umbilical artery	11.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N581	24.7	26	Renal dysplasia	12.4	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Elective abortion
N582	12.3	27	NT 4.7 mm	7.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N583	24.7	29	Cardiac defects, bilateral ventricles slightly asymmetric	7.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N584	29.9	33	Short femur and humerus	18.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N585	24.1	25	Cardiac defects	15.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N586	24.0	26	Hyperechogenic kidneys	5.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N587	22.4	29	Bright spots on the left ventricle, dilated left renal pelvis	7.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N588	24.1	27	Short nasal bone, nasal bone hypoplasia	9.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N589	24.0	32	Ventricular septal defect	14.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N590	23.4	31	Right aortic arch	8.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N591	25.3	37	Echogenic bowel, NF 6.3 mm	17.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N592	26.9	24	Double renal pelvis, nasal bone hypoplasia	26.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N593	25.0	33	Stenosis of pulmonary artery, ventricular septal defect	20.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N594	28.7	26	Dilatation of the renal pelvis, unilateral choroidal cyst	18.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N595	19.6	26	Atrial septal defect	7.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N596	23.0	30	NF 7.1 mm	14.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N597	23.0	35	NF 8.0 mm	15.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N598	22.6	33	Bright spots on the left ventricle	16.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N599	22.7	31	NF 7.3 mm	10.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N600	31.7	28	Small intestinal obstruction	23.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N601	16.7	28	Cystic hygroma	4.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N602	23.4	33	Renal agenesis, decreased head circumference	5.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N603	25.9	26	Horseshoe kidney, single umbilical artery	12.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N604	17.7	34	NT 5.0 mm	7.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N605	15.7	32	NT 4.7 mm	6.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N606	23.0	38	NF 6.4 mm	6.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N607	16.1	28	NF 7.7 mm	13.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N608	24.3	28	Cardiac defects, atrial septal defectsingle	11.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N609	22.3	27	Enlarged stomach bubble, polyhydramnios	9.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N610	22.7	32	NF 6.1 mm	12.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn

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Subject	Gestation age (wks)	Maternal age (yrs)	Indication	FF (%)	Comprehensive prenatal cfDNA screening result	Diagnostic testing					Specimen type	Pregnancy outcome
						CMA	Karyotyping	CNV-seq	NGS-SGD	WES		
N611	23.0	28	NF 9.0 mm	10.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N612	23.9	31	Pulmonary stenosis and insufficiency, small right ventricle	13.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N613	22.6	33	NF 6.8 mm, ventricular septal defect	10.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N614	22.6	34	NF 6.4 mm	9.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N615	13.7	27	Meningoencephalocele, enlarged bilateral rena	8.7	Low risk	-	Normal	Normal	Normal	-	Product of conception	Elective abortion
N616	24.9	26	Echogenic bowel, unossified nasal bone	8.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N617	22.7	30	Echogenic bowel	7.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N618	24.1	33	Short femur, microcephaly	13.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N619	20.3	30	Deviation of the thumb	11.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N620	22.1	39	NF 6.4 mm	15.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N621	25.3	23	Echogenic bowel	13.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N622	16.1	25	NT 4.5 mm, cleft lip and palate	19.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N623	22.6	29	NF 6.5 mm	7.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N624	22.6	31	Dilated coronary vein, ventricular septal defect	7.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N625	23.0	32	Urinary system abnormalities	14.9	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N626	17.4	30	NT 3.7 mm	12.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N627	17.6	32	NT 3.2 mm	7.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N628	23.3	39	NF 6.4 mm	8.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N629	30.7	26	Bilateral lateral ventriculomegaly	17.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N630	16.7	32	NT 3.3 mm	10.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N631	22.9	32	NF 6.4 mm	7.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N632	22.9	37	NF 7.4 mm	12.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N633	24.9	34	NF 7.1 mm	16.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N634	25.0	36	Lateral ventriculomegaly	12.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N635	23.1	36	NF 6.1 mm	24.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N636	22.9	38	NF 6.6 mm	15.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N637	22.9	31	Short nasal bone	11.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N638	21.9	30	Abnormal right atrium, butterfly vertebra	10.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N639	24.3	33	Hyperechogenic left ventricle, tricuspid regurgitation	19.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N640	22.3	30	NF 6.5 mm	12.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N641	28.1	33	Enlarged posterior fossa	21.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N642	22.6	36	Ventricular septal defect	16.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N643	26.7	33	Situs inversus viscerum, interrupted inferior vena cava	13.1	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N644	23.4	23	Small fetus	11.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N645	16.9	29	NT 5.3 mm	7.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N646	17.3	29	NT 4.6 mm	15.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N647	24.3	35	NF 6.5 mm	12.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N648	25.6	40	Kidney defect	11.4	Low risk	Normal	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N649	19.1	28	NT 5.1 mm	9.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Spontaneous abortion
N650	24.0	34	NF 6.5 mm, echogenic bowel	8.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N651	25.0	31	Small fetus, oligohydramnios, cardiomegaly	15.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N652	21.0	31	Right aortic arch, aberrant left subclavian artery	11.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N653	17.0	37	Small fetus	11.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N654	23.3	30	Dilated ventricle	9.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N655	21.4	27	Nasal bone absent	10.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N656	23.9	29	Right aortic arc, aberrant left subclavian artery	25.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N657	18.0	29	NT 3.1 mm	6.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N658	19.0	25	NT 3.5 mm	6.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N659	23.3	23	Cleft lip and palate	17.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N660	22.9	34	Cleft lip	7.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N661	25.3	31	Cleft lip	12.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N662	25.6	26	Anomalous intrahepatic portal vein	7.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N663	25.9	23	Cystic hygroma	10.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N664	17.1	28	Left choroidal cyst	12.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N665	24.9	32	Choroidal cyst	13.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N666	23.1	28	Right choroidal cyst	5.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N667	20.9	31	Bilateral choroidal cys	8.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N668	24.4	29	Left choroidal cyst	24.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N669	17.0	27	Bilateral choroidal cyst	16.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N670	23.0	31	Right aortic arch, left ductal arch, incomplete vascular ring	18.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N671	24.3	41	Echogenic abdominal cavity and bowel, celiac effusion	15.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N733	22.7	33	Pulmonary stenosis	9.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N734	22.7	34	Hypoplastic left heart, ventricular septal defect	15.1	Low risk	-	-	-	-	Normal	Product of conception	Elective abortion
N735	16.1	26	Cystic hygroma	11.6	Low risk	-	-	-	-	Normal	Amniocytes	Liveborn
N736	24.7	30	Suspected ectodermal dysplasia, and cleft lip/palate syndrome	18.6	Low risk	-	-	-	-	Normal	Product of conception	Elective abortion
N737	37.1	30	Cerebral white matter hypoplasia	30.1	Low risk	-	-	-	-	Normal	Product of conception	Liveborn
N738	29.0	38	Small fetus	7.9	Low risk	-	-	Normal	-	Normal	Amniocytes	Elective abortion
N739	24.3	39	Lobar holoprosencephaly	5.2	Low risk	-	Normal	Normal	-	Normal	Product of conception	Elective abortion
N740	22.0	32	Right renal pelvis separation	10.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N741	16.3	28	Bilateral cleft lip and palate, fingers and toes yndactyly	7.2	Low risk	-	-	Normal	Normal	Normal	Product of conception	Elective abortion
N742	27.6	31	Growth restriction	20.4	Low risk	-	-	Normal	Normal	Normal	Amniocytes	Liveborn
N743	32.0	22	Growth restriction	11.1	Low risk	-	-	Normal	Normal	Normal	Amniocytes	Liveborn
N744	25.1	23	Small fetus, reduced septum pellucidum	17.6	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N745	21.0	29	Multicystic left kidney dysplasia, ventricular septal defect	13.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N746	24.4	27	Digestive system abnormalities, anal atresia	12.8	Low risk	Normal	Normal	Normal	Normal	Normal	Amniocytes	Elective abortion
N747	17.4	29	NT 4.5 mm	10.2	Low risk	Normal	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N748	20.0	39	Growth restriction, bilateral lateral ventriculomegaly, echogenic bowel	4.0	Low risk	Normal	Normal	Normal	Normal	Normal	Amniocytes	Elective abortion
N749	23.1	34	Left hydronephrosis	19.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N750	27.3	34	Left multicystic kidney dysplasia	19.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	
N751	23.6	33	Diaphragmatic hernia	8.8	Low risk	Normal	Normal	Normal	Normal	Normal	Amniocytes	Elective abortion
N752	24.9	28	Decreased head circumference, abnormal skull morphology	12.2	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N753	16.9	30	NT 3.9 mm	8.8	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N754	24.6	29	Spinal abnormalities, lateral ventriculomegaly	10.4	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N755	26.3	26	Short femur, decreased head circumference, duplicated right kidney	16.6	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N756	24.7	28	Ventricular septal defect, narrow aortic valve annulus and isthmus	12.6	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Elective abortion
N757	25.7	34	Cardiac defects, increased echogenicity of the mitral valve	19.0	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N758	25.6	31	Multiple right renal cysts	10.2	Low risk	-	Normal	Normal	-	Normal	Product of conception	Liveborn
N759	18.9	33	NT 9.3 mm	11.1	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N760	23.1	26	Bilateral renal pelvis separation, omphalocele	18.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N761	20.6	32	Multicystic kidney dysplasia	8.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N762	28.0	34	Cerebellar vermis hypoplasia, polyhydramnios	15.0	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N763	31.9	28	Small fetus, decreased head circumference, short femur	11.0	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N764	26.4	27	Single umbilical artery, double left renal artery	12.1	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Elective abortion
N765	16.7	33	NT 5.4 mm	10.9	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N766	20.9	31	Kidney dysplasia, oligohydramnios	8.2	Low risk	-	-	Normal	Normal	-	Product of conception	Elective abortion
N767	25.3	35	Curved right femur	8.6	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N768	31.3	28	Decreased head and abdominal circumference	18.9	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Elective abortion
N769	23.7	31	Transposition of the heart great arteries	14.5	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N770	25.1	29	Bilateral lateral ventriculomegaly, berrant left subclavian artery	17.1	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	-
N771	20.4	30	Left multicystic kidney dysplasia	10.0	Low risk	Normal	Normal	Normal	Normal	Normal	Amniocytes	-
N772	23.3	31	Flat nose	17.2	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N773	17.7	37	NT 4.4 mm	17.8	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N774	28.9	34	Left lateral ventriculomegaly, aberrant right subclavian artery	19.5	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N775	22.6	39	Aberrant right subclavian artery, echogenic bowel and ventricle	9.2	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	-
N776	24.0	37	Bilateral clubfoot, bright spots on the left ventricle	7.6	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	-
N777	24.4	27	Clubfoot, bright spots on the left ventricle	13.6	Low risk	-	Normal	Normal	Normal	Normal	Amniocytes	Liveborn
N778	18.0	37	Standard prenatal cfDNA screening high risk	14.5	Low risk	-	-	Normal	-	-	Amniocytes	Liveborn
N779	18.3	31	Standard prenatal cfDNA screening high risk	10.1	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N780	23.0	33	Standard prenatal cfDNA screening high risk	8.8	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N781	17.4	35	Standard prenatal cfDNA screening high risk	8.9	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N782	21.3	28	Standard prenatal cfDNA screening high risk	6.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N783	17.0	32	Standard prenatal cfDNA screening high risk	8.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N784	20.3	30	Standard prenatal cfDNA screening high risk	8.7	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N785	19.4	30	Standard prenatal cfDNA screening high risk	4.5	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N786	16.0	28	Standard prenatal cfDNA screening high risk	17.0	Low risk	Normal	-	Normal	Normal	-	Amniocytes	Elective abortion
N787	21.6	38	Standard prenatal cfDNA screening high risk	7.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N788	26.0	33	Standard prenatal cfDNA screening high risk	8.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	-
N789	26.9	25	Standard prenatal cfDNA screening high risk	17.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N790	19.1	36	Standard prenatal cfDNA screening high risk	18.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N791	19.6	35	Standard prenatal cfDNA screening high risk	12.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N792	25.6	33	Standard prenatal cfDNA screening high risk	13.1	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N793	18.7	29	Standard prenatal cfDNA screening high risk	15.0	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn

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Extended Data Table 6 (continued) | The diagnostic testing results and pregnancy outcomes of pregnancies with negative prenatal cfDNA screening results

Subject	Gestation age (wks)	Maternal age (yrs)	Indication	FF (%)	Comprehensive prenatal cfDNA screening result	Diagnostic testing					Specimen type	Pregnancy outcome
						CMA	Karyotyping	CNV-seq	NGS-SGD	WES		
N794	21.3	30	Standard prenatal cfDNA screening high risk	13.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N795	22.7	25	Standard prenatal cfDNA screening high risk	13.1	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N796	19.9	34	Standard prenatal cfDNA screening high risk	14.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N797	21.4	35	Standard prenatal cfDNA screening high risk	11.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N798	17.1	23	Standard prenatal cfDNA screening high risk	18.0	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N799	21.0	34	Standard prenatal cfDNA screening high risk	12.2	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N800	21.6	32	Standard prenatal cfDNA screening high risk	12.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Elective abortion
N801	34.6	29	Standard prenatal cfDNA screening high risk	35.4	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N802	17.3	31	Standard prenatal cfDNA screening high risk	8.0	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N803	18.0	38	Standard prenatal cfDNA screening high risk	7.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N804	24.0	29	Standard prenatal cfDNA screening high risk	31.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N805	20.0	38	Standard prenatal cfDNA screening high risk	7.1	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N806	18.0	39	Standard prenatal cfDNA screening high risk	7.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N807	19.4	32	Standard prenatal cfDNA screening high risk	6.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N808	20.9	31	Standard prenatal cfDNA screening high risk	13.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N809	16.9	33	Standard prenatal cfDNA screening high risk	9.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N810	19.3	30	Standard prenatal cfDNA screening high risk	19.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N811	24.6	31	Standard prenatal cfDNA screening high risk	8.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N812	18.0	24	Standard prenatal cfDNA screening high risk	9.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N813	17.4	38	Standard prenatal cfDNA screening high risk	10.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N814	18.9	31	Standard prenatal cfDNA screening high risk	12.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N815	22.4	29	Standard prenatal cfDNA screening high risk	11.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N816	22.3	29	Standard prenatal cfDNA screening high risk	7.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N817	20.3	25	Standard prenatal cfDNA screening high risk	9.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N818	19.3	30	Standard prenatal cfDNA screening high risk	12.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N819	19.7	28	Standard prenatal cfDNA screening high risk	10.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N820	26.0	34	Standard prenatal cfDNA screening high risk	19.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N821	22.3	35	Standard prenatal cfDNA screening high risk	12.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N822	19.7	22	Standard prenatal cfDNA screening high risk	16.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N823	20.4	35	Standard prenatal cfDNA screening high risk	9.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N824	19.0	36	Maternal serum screening high risk	18.4	Low risk	-	Normal	-	-	-	Amniocytes	Liveborn
N825	17.4	39	Maternal serum screening high risk	9.5	Low risk	-	-	Normal	-	-	Amniocytes	Liveborn
N826	16.1	28	Maternal serum screening high risk	13.6	Low risk	-	Normal	Normal	-	-	Amniocytes	-
N827	18.4	31	Maternal serum screening high risk	10.1	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N828	17.0	35	Maternal serum screening high risk	14.1	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N829	19.7	44	Maternal serum screening high risk	7.8	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N830	18.7	29	Maternal serum screening high risk	9.4	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N831	18.6	36	Maternal serum screening high risk	6.6	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N832	19.3	34	Maternal serum screening high risk	8.0	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N833	18.1	40	Maternal serum screening high risk	14.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N834	18.0	33	Maternal serum screening high risk	18.7	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N835	18.4	36	Maternal serum screening high risk	17.1	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N836	19.4	31	Maternal serum screening high risk	8.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N837	18.7	28	Maternal serum screening high risk	11.7	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N838	19.0	25	Maternal serum screening high risk	12.3	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N839	20.4	43	Maternal serum screening high risk	3.6	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N840	18.0	34	Maternal serum screening high risk	7.7	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N841	22.0	41	Maternal serum screening high risk	11.1	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N842	19.9	31	Maternal serum screening high risk	9.0	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N843	19.0	37	Maternal serum screening high risk	8.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N844	31.7	33	Maternal serum screening high risk	24.9	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N845	19.9	35	Maternal serum screening high risk	9.6	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N846	18.6	30	Maternal serum screening high risk	14.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N847	18.7	41	Maternal serum screening high risk	11.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N848	19.3	27	Maternal serum screening high risk	6.1	Low risk	Normal	-	Normal	Normal	-	Amniocytes	-
N849	18.4	32	Maternal serum screening high risk	7.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N850	21.0	32	Maternal serum screening high risk	10.0	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N851	25.7	27	Maternal serum screening high risk	19.4	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N852	21.4	32	Maternal serum screening high risk	5.8	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N853	17.9	28	Maternal serum screening high risk	11.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N854	19.6	22	Maternal serum screening high risk	15.5	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N855	18.7	31	Maternal serum screening high risk	23.2	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N856	19.0	32	Maternal serum screening high risk	17.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N857	18.9	33	Maternal serum screening high risk	15.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N858	18.1	41	Maternal serum screening high risk	6.0	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N859	19.4	33	Maternal serum screening high risk	6.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N860	20.3	21	Maternal serum screening high risk	15.7	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N861	19.0	35	Maternal serum screening high risk	4.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N862	28.9	30	Maternal serum screening high risk	21.2	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N863	16.0	38	Maternal serum screening high risk	13.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N864	17.6	34	Maternal serum screening high risk	14.8	Low risk	-	-	Normal	Normal	-	Amniocytes	-
N865	20.4	22	Maternal serum screening high risk	17.8	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N866	18.0	34	Maternal serum screening high risk	13.4	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N867	19.0	35	Maternal serum screening high risk	5.6	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N868	22.3	41	Maternal serum screening high risk	9.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N869	24.0	33	Maternal serum screening high risk	18.2	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N870	18.6	35	Maternal serum screening high risk	15.9	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N871	20.0	40	Maternal serum screening high risk	11.5	Low risk	-	-	Normal	Normal	-	Amniocytes	Liveborn
N872	18.0	26	Maternal serum screening high risk	13.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N873	18.1	31	Maternal serum screening high risk	8.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N874	18.6	27	Maternal serum screening high risk	5.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N875	20.9	31	Maternal serum screening high risk	11.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N876	20.1	29	Maternal serum screening high risk	4.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N877	17.4	24	Maternal serum screening high risk	18.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N878	16.9	32	Maternal serum screening high risk	12.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N879	19.0	33	Maternal serum screening high risk	8.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N880	17.4	26	Maternal serum screening high risk	15.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N881	18.9	29	Maternal serum screening high risk	7.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N882	19.1	25	Maternal serum screening high risk	9.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N883	22.0	33	Maternal serum screening high risk	7.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N884	17.7	42	Maternal serum screening high risk	9.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N885	17.7	32	Maternal serum screening high risk	12.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N886	18.7	46	Maternal serum screening high risk	7.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N887	17.9	33	Maternal serum screening high risk	12.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N888	17.6	40	Maternal serum screening high risk	9.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N889	16.9	38	Maternal serum screening high risk	14.0	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N890	17.0	31	Maternal serum screening high risk	9.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N891	19.7	21	Maternal serum screening high risk	14.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N892	20.6	30	Maternal serum screening high risk	12.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N893	17.0	36	Maternal serum screening high risk	14.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N894	17.6	36	Maternal serum screening high risk	11.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N895	19.0	32	Maternal serum screening high risk	8.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N896	17.1	42	Maternal serum screening high risk	8.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N897	16.0	29	Maternal serum screening high risk	24.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N898	19.0	38	Maternal serum screening high risk	13.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N899	16.0	26	Maternal serum screening high risk	13.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N900	20.3	30	Maternal serum screening high risk	11.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N901	18.6	34	Maternal serum screening high risk	5.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N902	20.7	32	Maternal serum screening high risk	7.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N903	20.0	37	Maternal serum screening high risk	10.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N904	19.1	31	Maternal serum screening high risk	11.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N905	21.7	38	Maternal serum screening high risk	9.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N906	19.0	33	Maternal serum screening high risk	10.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N907	18.3	29	Maternal serum screening high risk	12.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N908	17.0	24	Maternal serum screening high risk	3.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N909	18.3	45	Maternal serum screening high risk	11.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N910	19.4	38	Maternal serum screening high risk	4.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N911	23.3	34	Maternal serum screening high risk	9.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N912	16.0	32	Maternal serum screening high risk	7.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N913	20.0	32	Maternal serum screening high risk	9.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N914	20.1	40	Maternal serum screening high risk	8.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N915	20.1	27	Maternal serum screening high risk	13.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn

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Extended Data Table 6 (continued) | The diagnostic testing results and pregnancy outcomes of pregnancies with negative prenatal cfDNA screening results

Subject	Gestation age (wks)	Maternal age (yrs)	Indication	FF (%)	Comprehensive prenatal cfDNA screening result	Diagnostic testing					Specimen type	Pregnancy outcome
						CMA	Karyotyping	CNV-seq	NGS-SGD	WES		
N916	17.0	39	Maternal serum screening high risk	9.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N917	18.6	30	Maternal serum screening high risk	15.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N918	20.6	32	Maternal serum screening high risk	10.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N919	17.6	38	Maternal serum screening high risk	7.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N920	24.0	36	Maternal serum screening high risk	5.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N921	20.0	32	Maternal serum screening high risk	12.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N922	17.4	38	Maternal serum screening high risk	5.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N923	17.1	33	Maternal serum screening high risk	13.8	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N924	18.0	37	Maternal serum screening high risk	8.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N925	18.6	36	Maternal serum screening high risk	16.7	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N926	19.0	36	Maternal serum screening high risk	7.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N927	18.9	32	Maternal serum screening high risk	18.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N928	18.0	32	Maternal serum screening high risk	13.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N929	19.0	37	Maternal serum screening high risk	6.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N930	21.7	38	Maternal serum screening high risk	6.1	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N931	20.0	42	Maternal serum screening high risk	10.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N932	17.7	38	Maternal serum screening high risk	9.6	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N933	20.0	40	Maternal serum screening high risk	4.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N934	19.9	35	Maternal serum screening high risk	10.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N935	19.1	30	Maternal serum screening high risk	3.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N936	19.9	26	Maternal serum screening high risk	5.4	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Elective abortion
N937	17.6	28	Clinical history suggestive of genetic conditions	8.7	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N938	18.6	38	Clinical history suggestive of genetic conditions	12.4	Low risk	-	Normal	Normal	-	-	Amniocytes	Liveborn
N939	20.0	31	Clinical history suggestive of genetic conditions	24.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N940	18.6	31	Clinical history suggestive of genetic conditions	9.3	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N941	19.4	38	Clinical history suggestive of genetic conditions	5.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N942	19.0	37	Clinical history suggestive of genetic conditions	3.6	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N943	19.7	29	Clinical history suggestive of genetic conditions	10.7	Low risk	Normal	Normal	Normal	Normal	-	Amniocytes	Liveborn
N944	16.4	38	Clinical history suggestive of genetic conditions	6.5	Low risk	-	Normal	Normal	Normal	-	Amniocytes	-
N945	16.3	35	Clinical history suggestive of genetic conditions	10.9	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N946	25.4	28	Clinical history suggestive of genetic conditions	12.2	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn
N947	16.7	27	Clinical history suggestive of genetic conditions	14.3	Low risk	-	Normal	Normal	Normal	-	Amniocytes	Liveborn

FF: fetal fraction. CMA: chromosome microarray analysis. CNV-seq: next-generation sequencing based chromosomal copy-number variation analysis. NGS-SGD: next-generation sequencing panel for all targeted single-gene disorders. WES: whole-exome sequencing.

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Extended Data Table 7 | Pregnancy outcomes in participants with positive and negative diagnostic testing results

Pregnancy outcomes	Negative cases – no. (%)	Positive cases – no. (%)	Positive cases with ultrasound abnormalities – no. (%)	Positive cases with other indications – no. (%)
Live birth	612 (64.2)	11 (8.0)	4 (4.0)	7 (18.9)
Elective abortion	162 (17.0)	106 (77.4)	82 (82.0)	24 (64.9)
Spontaneous abortion	1 (0.1)	1 (0.7)	1 (1.0)	0
Unknown[1]	178 (18.7)	19 (13.9)	13 (13.0)	6 (16.2)
Total[2]	953	137	100	37

https://doi.org/10.1038/s41591-023-02774-x

Extended Data Table 8 | Parental age and the occurrence of different genetic variants

Subjects	Mean maternal age, years (number of subjects)	*P*-value[1]	Mean paternal age, years (number of subjects)	*P*-value
True positive for autosome aneuploidies	32.8 (61)	0.005	34.4 (42)	0.037
True negative for autosome aneuploidies	30.7 (1,015)		32.5 (781)	
True positive sex chromosome aneuploidies	30.9 (28)	0.943	33.7 (20)	0.512
True negative for sex chromosome aneuploidies	30.8 (1,052)		32.6 (804)	
True positive for microdeletions	30.9 (9)	0.965	32.4 (7)	0.887
True negative for microdeletions	30.8 (1,062)		32.6 (813)	
True positive for monogenic conditions	31.0 (37)	0.658	33.2 (23)	0.486
True negative for monogenic conditions	30.8 (966)		32.5 (741)	

[1]T-test was performed with a two-tailed test, and multiple comparisons were not conducted.

nature portfolio

Corresponding author(s): Jinglan Zhang

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Microsoft Excel was used for the clinical data collection.
Data analysis	Customized computing code used in this study is available at https://github.com/Jinglan1/NIPS2/. Raw FASTQ were filtered and UMI preprocessed using FASTP 0.21.0, https://github.com/OpenGene/fastp. The clean FASTQ files were aligned to hg38 human reference using BWA 0.7.17-r1188, https://github.com/lh3/bwa and then sorted by Samtools 1.9, https://github.com/samtools/samtools/releases/. Consensus BAM files were generated by Gencore 0.15.0 and then finalized by BaseRecalibrator and ApplyBQSR GATK 4.1.8.0 followed by variant calling, https://gatk.broadinstitute.org. Raw variants were annotated by Annovar v2019-10-24, https://annovar.openbioinformatics.org/. Chromosomal microarray analysis was performed using ChAS software 3.1.

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The demographic data, clinical history, prenatal cfDNA screening, diagnostic test results, and the diagnostic test methodologies of all 1,090 participants in the final cohort are within the paper and the Extended Data. All the pathogenic single-gene variants and the key phenotypes of the subjects are available at the ClinVar database at https://www.ncbi.nlm.nih.gov/clinvar/submitters/508997/. The raw data files for all 1,090 participants are securely stored in an environment compliant with patients' privacy protection regulations within our laboratory and will be maintained for a minimum of ten years following publication. Access to these raw data files, unfiltered cfDNA gene sequencing data (VCF files) and locus-specific diagnostic sequencing results, is available upon request from the corresponding author, J.Z. (jinglanzhang@fudan.edu.cn or jinglanzhang@foxmail.com). This process is to assure that patients' data privacy will be safeguarded, and that the data will be utilized exclusively for non-commercial academic research purposes. All requests for the data access must originate from an academic institution and be accompanied by verifiable affiliation (e.g., a publicly accessible research investigator profile on the institution's website). Upon receipt of a qualified request, it will undergo review by a Data Privacy Committee (DPC), composed of two senior investigators from the study and an external reviewer, to verify that the data will be used exclusively for non-commercial, academic research purposes. After DPC approval, the execution of a Data Transfer Agreement is required which will explicitly stipulate non-disclosure to third party and that the data is to be used solely for non-commercial, academic research activities. Qualified requests will be processed within a three-week time frame. The hg38 reference genome sequence can be obtained at https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001405.40/.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Not applicable.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable.
Population characteristics	The final cohort consisted of 1,090 qualified participants. The mean maternal age of all qualified participants was 30.8 years. The proportion of women carrying pregnancies at the gestational ages of 12-18 weeks, 19-24 weeks, and ≥25 weeks was 28.9%, 39.8%, and 31.3%. Among them, 876 (80.4%) had fetal ultrasound anomalies, 116 (10.6%) had abnormal maternal serum screening results, 86 (7.9%) had high-risk results in standard prenatal cfDNA screening for chromosomal conditions, and 12 (1.1%) had a previous pregnancy history suggesting an increased risk for fetal genetic conditions. This population was representative for pregnancies with elevated risks of fetal genetic conditions commonly seen in prenatal clinical setting. The limitation of this study is its focus on pregnancies already identified as high-risk for fetal genetic conditions. In the general population, the likelihood of these genetic conditions is expected to be much lower than in the high-risk group. This difference could influence the test's its positive predictive values (PPVs), in detecting ultra-rare genetic conditions in a broader, lower-risk population.
Recruitment	Between April 24, 2021, and September 10, 2022, 1,191 sequentially identified pregnant women were enrolled and followed up from three maternity hospitals in different provinces of China. The recruitment was performed according to a previously published study protocol (DOI: 10.1136/bmjopen-2021-053617). The trial registration number is ChiCTR2100045739.
Ethics oversight	This study had been reviewed and approved by the internal review board at the Obstetrics and Gynecology Hospital of Fudan University (2020-178). This clinical study led by the Obstetrics and Gynecology Hospital of Fudan University has received the approval for the collection of human genetic resources in China from the Ministry of Science and Technology (MOST) of China (2021-CJ0599). The trial registration number was ChiCTR2100045739 with a published study protocol.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Before the start of this study, we performed a power analysis and planned to enroll at least 1,000 participants from whom we expected to detect at least 25 cases affected by the targeted chromosomal and monogenic conditions. This estimation was based on the detection rate

among pregnancies with similar indications. The sample size in this study would allow a probability of 95% or above to observe a possible measuring error at the case level for both the chromosomal and monogenic conditions.

Data exclusions

Of the 101 excluded cases, 71 had no diagnostic test results available for fetal germline variants, 15 had maternal variants in targeted genomic regions interfering with fetal assessment, eight did not meet the sequencing depth requirements for the prenatal cfDNA screening assay, and seven failed quality control for singleton pregnancy due to multiple gestation or sample contamination. The final cohort consisted of 1,090 (91.5%) qualified participants whose pregnancies underwent further analyses, in which results derived from their comprehensive cfDNA screening and diagnostic testing were compared.

Replication

Participants were 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 number of subjects from each hospital were collected given essentially equal participant availability to avoid potential population stratification.

Randomization

This was an observational study to investigate the clinical validity and detection rate of genetic conditions for a prenatal screening test. The patient cohort was consisted of sequentially identified pregnant women from three maternity hospitals in different provinces of China. In addition, the cohort included a large variety of fetal anomalies instead of targeted conditions (Table 1), which made this study more generalizable to uncover the detectability of the prenatal cfDNA screening for genetic conditions.

Blinding

This was a prospective, multicenter cohort study comparing the screening and diagnostic testing results of a comprehensive prenatal cfDNA screening covering three of the most frequent causes of human genetic condition: aneuploidies, microdeletions, and monogenic conditions. The diagnostic results for each case were not revealed until the screening test was finalized in order to evaluate the clinical performance of the prenatal cfDNA screening test.

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☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

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Clinical trial registration

The study registration number: ChiCTR2100045739 (https://www.chictr.org.cn/showprojEN.html?proj=125206).

Study protocol

The study was performed according to a previously published protocol (DOI: 10.1136/bmjopen-2021-053617).

Data collection

Between April 24, 2021, and September 10, 2022, 1,191 sequentially identified pregnant women were enrolled and followed up from three maternity hospitals in different provinces of China and the clinical data regarding their pregnancies were collected.

Outcomes

The outcomes of the study were the clinical validity of an expanded prenatal cfDNA screening and its detection rate for different types of genetic conditions causing fetal anomalies. Complete results for both screening and diagnostic testing (i.e., testing on chorionic villus sampling, amniocentesis, products of conception, etc.) were collected and compared for all qualified participants. The clinical validity was measured by calculating the screening test sensitivity, specificity, positive predictive value, negative predictive value, and the area under the receiver-operating-characteristic (ROC) curve (AUC). Only women with confirmatory genetic testing were included in the results and those without any genetic diagnostic testing results were excluded. The detection rates of a diagnostic genetic variant associated with aneuploidies, microdeletions, and monogenic conditions were measured for the entire cohort and with respect to different fetal anomalies. The study also aimed to collect the pregnancy outcome data of the participants by reviewing medical records, which included miscarriages, elective abortions, stillbirths, and live-birth deliveries. When medical records of pregnancy outcomes were not available in the participating hospitals, participants were contacted by phone up to three attempts and up until six weeks after the expected delivery date. Pregnancy outcomes and clinical examination results were evaluated to examine if they were consistent with the genetic diagnosis.

Plants

Seed stocks	Not applicable.
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