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Early second-trimester plasma cell free DNA levels with subsequent risk of pregnancy complications

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ABSTRACT

Objective: To investigate the association between cfDNA levels measured during non-invasive prenatal testing (NIPT) and the risk of pregnancy complications in a Chinese population.

Methods: This was a retrospective cohort study of 831 pregnant women who underwent NIPT at 12–22 weeks of gestation. Maternal plasma cfDNA levels and pregnancy outcomes were obtained from NIPT Screening System and hospitalization records, respectively. Logistic regression analysis was performed to investigate the relationship between cfDNA levels and pregnancy complications (after adjusting for confounding factors).

Results: Maternal cfDNA levels were significantly higher in women diagnosed with intrahepatic cholestasis of pregnancy (ICP) and preeclampsia (PE) compared to pregnant women with non-pregnancy complications (NPC) (median cfDNA 7.07, 6.42 vs. 5.99 ng/mL). Increase in cfDNA levels were associated with an increased risk for ICP (adjusted-OR = 1.20, 95% CI: 1.07–1.34) and PE (adjusted-OR = 1.14, 95% CI: 1.02–1.26). In addition, increase in cfDNA levels were associated with risk of GDM, and was dependent on maternal age (maternal age ≥ 35 years: adjusted-OR = 1.16, 95% CI: 1.04–1.29; maternal age < 35 years: adjusted-OR = 0.85, 95% CI: 0.73–0.99).

Conclusion: Maternal plasma cfDNA levels measured during NIPT are associated with pregnancy complications (ICP, PE and GDM). Maternal age may be an important effect modifier for the association between plasma cfDNA levels and GDM.

1. Introduction

Non-invasive prenatal testing (NIPT) using maternal plasma cfDNA has been widely used to screen common chromosomal aneuploidies [1–3]. Several studies have demonstrated that maternal plasma or serum cell free DNA (cfDNA) levels in women with fetal growth restriction, preeclampsia (PE) and HELLP syndrome are higher compared to healthy pregnant women [4–9]. Maternal plasma cfDNA levels have been shown to be a suitable biomarker to predict pregnancy complications, including PE and intrauterine growth restriction [10,11]. However, maternal cfDNA levels measured at 11–13 and 20–24 weeks of gestation were not associated with eclampsia [12]. To date, only a few reports have been published on the relationship between maternal cfDNA levels during the early-second trimester of pregnancy and specific pregnancy complications, such as gestational diabetes mellitus (GDM) and intrahepatic cholestasis of pregnancy (ICP).

GDM is characterized by hyperglycemia and hyperinsulinemia and affects about 7.4–24.5% of all pregnancies in China [13,14]. In addition to being at high risk for adverse pregnancy outcomes (e.g., hypertension, preeclampsia, premature labor and fetal macrosomia), pregnant women with GDM are at a greater risk of developing type 2 diabetes and cardiovascular diseases later in life [15,16]. In addition, babies from the affected mother are more likely to develop childhood obesity and other metabolic disorders in adolescence and early adult life [17]. More importantly, female babies born to mothers with GDM are prone to develop GDM during their own pregnancies [18]. Risk assessments

Abbreviations: cfDNA, Cell free DNA; NIPT, Non-invasive prenatal testing; ICP, Intrahepatic cholestasis of pregnancy; PE, Preeclampsia; NPC, Non-pregnancy complication; GDM, Gestational diabetes mellitus; PIH, Pregnancy-induced hypertension; BMI, Body mass index; LGA, Large for gestational age; SGA, Small for gestational age; PTB, Preterm birth; FTB, Full term birth.

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Table 1
Maternal characteristics, neonatal outcomes and maternal cfDNA levels in the study populations according to pregnancy complications[a].

	NPC	GDM	ICP	PE	PIH
Subjects [N(%)]	630 (75.81)	111 (13.36)	39 (4.69)	52 (6.26)	14 (1.68)
Maternal age at delivery (years)	33 (28–36)	35 (31–37)**	31 (27–37)	35 (32–36)	32 (26–36)
BMI at NIPT (kg/m2)[b]	22.4 (20.8–24.4)	23.4 (21.5–25.9)**	22.1 (20.7–25.1)	24.8 (22.1–27.0)**	24.2 (22.0–25.6)*
BMI at delivery (kg/m2)	27.4 (25.4–29.4)	27.8 (25.4–30.0)	27.3 (24.5–31.0)	29.5 (26.5–32.8)**	31.3 (28.2–32.0)**
Gestational weight gain (kg)[c]	12 (10–15)	10 (7–14)**	13 (10–17)	14 (9–16)	17 (16–20)**
Gravidity	2 (1–3)	3 (1–3)	2 (1–3)	2 (1–3)	2 (1–3)
< 3 [N(%)]	362 (57.5)	55 (49.6)	26 (66.7)	32 (61.5)	10 (71.4)
≥3 [N(%)]	268 (42.5)	56 (50.4)	13 (33.3)	20 (38.5)	4 (28.6)
Parity	2 (1–2)	2 (1–2)	1 (1–2)*	2 (1–2)	1 (1–2)
No child [N(%)]	270 (42.9)	50 (45.1)	24 (61.5)*	23 (44.2)	9 (64.3)
≥1 child [N(%)]	360 (57.1)	61 (54.9)	15 (38.5)*	29 (55.8)	5 (35.7)
Gestational age at NIPT (day)	125 (119–131)	122 (116–129)*	123 (120 − 131)	125 (119–132)	126 (120 − 130)
Gestational age at delivery (week)	39 (38–39)	38 (38–39)*	38 (38–39)	38 (37–39)**	39 (39–39)
Systolic BP at delivery (mmHg)	116 (110–126)	119 (110–127)	121 (113–129)	122 (117–125)**	147 (141–149)**
Diastolic BP at delivery (mmHg)	70 (70–77)	72 (70–78)	72 (70–79)	91 (88–100)**	90 (86–90)**
Delivery mode					
Vaginal delivery	224 (35.6)	32 (28.8)	11 (28.2)	17 (32.7)	4 (28.6)
Cesarean section	406 (64.4)	79 (71.2)	28 (71.8)	35 (67.3)	10 (71.4)
PTB	21 (3.3)	5 (4.5)	3 (7.7)	8 (15.4)**	0
FTB	609 (96.7)	106 (95.5)	36 (92.3)	44 (84.6)**	14 (100.00)
Infant's sex					
Female	321 (51.0)	56 (50.5)	19 (48.7)	22 (42.3)	8 (57.1)
Male	309 (49.0)	55 (49.5)	20 (51.3)	30 (57.7)	6 (42.9)
Fetal birth weight (gram)	3405 (3160–3680)	3500 (3155–3800)	3480 (3040–3705)	3295 (2885–3653)**	3755 (3058–3980)
< 2500	11 (1.8)	4 (3.6)*	3 (7.7)*	10 (19.2)**	1 (7.1)*
2500–4000	575 (91.3)	91 (82.0)*	32 (82.1)*	39 (75.0)**	10 (71.4)*
> 4000	44 (6.9)	16 (14.4)*	4 (10.2)*	3 (5.8)**	3 (21.5)*
Weight for gestational age					
SGA	38 (6.0)	2 (1.80)**	2 (5.1)	8 (15.4)*	2 (14.3)*
AGA	475 (75.4)	76 (68.5)**	28 (71.8)	35 (67.3)*	7 (50.0)*
LGA	117 (18.6)	33 (29.7)**	9 (23.1)	9 (17.3)*	5 (35.7)*
Maternal plasma cfDNA (ng/mL)	5.99 (4.58–7.49)	5.95 (4.69–8.29)	7.07 (5.46–8.45)**	6.42 (4.94–9.35)**	6.44 (4.94–7.51)

Notes: Data was presented as median (IQR) or N(%). *$P < 0.05$, **$P < 0.01$, compared with NPC group.
Abbreviations: IQR, interquartile range; cfDNA, cell free DNA; NPC, non–pregnancy complication; GDM, gestational diabetes mellitus; ICP intrahepatic cholestasis of pregnancy; PE, preeclampsia; PIH, pregnancy–induced hypertension; BMI, body mass index; NIPT, Non invasive prenatal test; BP, blood pressure; PTB, pre–term birth; FTB, full–term birth; SGA/AGA/LGA small/appropriate/large for gestational age.
[a] 15 cases had more than one kind of complications.
[b] 13 cases missing maternal height or weight at NIPT. [c]Gestational weight gain in pregnancy from NIPT to delivery.

and early lifestyle modifications can reduce the occurrence of GDM [19].

ICP is a pregnancy-specific liver disease that occurs during the late second and third trimester of pregnancy. It is characterized by itching and jaundice. It contributes to an increased risk for adverse perinatal outcomes in the fetus and the subsequent development of hepatobiliary disease in the mother [20]. Abnormal bile duct transport and reduced bile acid elimination plays an important role in the ICP etiology, however its pathophysiology has not been fully elucidated. To our knowledge, only a few reports of maternal cfDNA levels associating with ICP or GDM during the first and second trimester of pregnancy have been published. The aim of our study was to examine the relationship between maternal cfDNA levels measured during NIPT with major pregnancy complications, such as GDM, ICP, PE and pregnancy-induced hypertension (PIH). A retrospective observational cohort was used to determine whether maternal cfDNA levels could be used as a assessment biomarker for these complications in the Chinese population.

2. Materials and methods

2.1. Study population

This was a retrospective observational cohort study of 831 consecutive pregnant women who underwent NIPT at 12–22 weeks' gestation in Changzhou Maternity and Child Health Care Hospital from October 2015 to December 2016. The study was approved by the ethics committee of Changzhou Maternity and Child Health Care Hospital (No. ZD201803) prior to the data collection. Written informed consent was obtained from each participant in our retrospective study. All clinical data was analyzed with patient identification anonymized. Inclusion criteria of the cohort were as follows: 1) pregnant women between 18 and 50 years old; 2) were between 12 and 22 weeks of gestation; 3) had negative NIPT results and gave birth in our hospital; 4) singleton pregnancy, live birth without birth defects. Exclusion criteria were: 1) missing or incomplete medical records; 2) preexisting illnesses prior to pregnancy: diabetes mellitus (type 1 or 2), chronic hypertension, thyroid diseases, chronic heart, liver and kidney diseases, immune rheumatic disease or thyroid diseases and syphilis prior to pregnancy. None of the study participants smoked or consumed alcohol during pregnancy. Thirty-three of 893 observational participants were excluded because of twin pregnancy. Fifteen women were exclude due to newborns having congenital malformations (n = 7), medical abortion (n = 3), stillbirth (n = 3), and pre-gestational diseases (chronic hypertension, n = 1; syphilis, n = 1). 831 eligible women with singleton pregnancy met the study inclusion criteria. Maternal age, body mass index (BMI) and cfDNA levels at NIPT were obtained from our prenatal screening database. Maternal and neonatal characteristics were obtained from our hospital database, which included maternal BMI at delivery, gravidity, parity, past medical history, tobacco and alcohol use, pregnancy complications, delivery method, fetal birth weight and gender of the baby.

Table 2
Maternal characteristics and neonatal outcomes in the study populations according to the categories of maternal plasma cfDNA levels.

	Low plasma cfDNA	High plasma cfDNA	*P* value
	Tertile 1–2 (≤6.94 ng/mL)	Tertile 3 (> 6.94 ng/mL)	
Subjects [N(%)]	552 (66.4)	279 (33.6)	
Maternal age at delivery (years)	33 (28–36)	34 (29–37)*	0.016
BMI at NIPT (kg/m2)[a]	22.5 (20.8–24.4)	22.8 (21.1–25.4)*	0.021
BMI at delivery (kg/m2)	27.5 (25.3–29.4)	27.7 (25.7–30.1)*	0.038
Gestational weight gain (kg)[b]	12.0 (9.9–15.1)	12.0 (9.5–15.0)	0.605
Gravidity	2 (1–3)	2 (1–3)	0.727
< 3 [N(%)]	323 (58.5)	153 (54.8)	0.312
≥3 [N(%)]	229 (41.5)	126 (45.2)	
Parity	2 (1–2)	2 (1–2)	0.828
No child [N(%)]	242 (43.8)	124 (44.4)	0.868
≥1 child [N(%)]	310 (56.2)	155 (55.6)	
Gestational age at NIPT (day)	124 (119–131)	124 (119–131)	0.769
Gestational age at delivery (week)	39 (38–39)	39 (38–39)	0.443
Systolic BP at delivery (mmHg)	116 (110–128)	120 (110−130)**	0.004
Diastolic BP at delivery (mmHg)	70 (70–78)	73 (70–78)**	0.005
Delivery mode			
Vaginal delivery	202 (36.6)	82 (29.4)*	0.039
Cesarean section	350 (63.4)	197 (70.6)*	
PTB	20 (3.6)	16 (5.7)	0.158
FTB	532 (96.4)	263 (94.3)	
Pregnancy compilation			
GDM	73 (13.2)	38 (13.6)	0.605
ICP	18 (3.3)	21 (7.5)**	0.004
PE	28 (5.1)	24 (8.6)*	0.033
PIH	9 (1.6)	5 (1.8)	0.753
Infant's sex			
Female	277 (50.2)	141 (50.5)	0.923
Male	275 (49.8)	138 (49.5)	
Fetal birth weight (gram)	3410 (3150–3710)	3420 (3155–3700)	0.829
< 2500	16 (2.9)	12 (4.3)	0.502
2500–4000	492 (89.1)	243 (87.1)	
> 4000	44 (8.0)	24 (8.6)	
Weight for gestational age			
SGA	36 (6.5)	16 (5.7)	0.823
AGA	401 (72.6)	208 (74.6)	
LGA	115 (20.8)	55 (19.7)	

Notes: Data was presented as median (IQR) or N(%). *$P < 0.05$, **$P < 0.01$, compared with low plasma cfDNA group.
Abbreviations: IQR, interquartile range; cfDNA, cell free DNA; BMI, body mass index; NIPT, Non invasive prenatal test; BP, blood pressure; PTB, pre–term birth; FTB, full-term birth; GDM, gestational diabetes mellitus; ICP, intrahepatic cholestasis of pregnancy; PE, Preeclampsia; PIH, pregnancy-induced hypertension; SGA/AGA/LGA, small/appropriate/large for gestational age.
[a] 13 cases missing maternal height or weight at NIPT.
[b] Gestational weight gain in pregnancy from NIPT to delivery.

2.2. Cell free DNA extraction and sequencing

Maternal whole blood sample (10 mL) was collected using EDTA-K2 tubes (BD Vacutainer 367,525, Becton, Dickinson & Co. UK). Plasma was obtained by whole blood centrifugation at 1600*g* for 10 min at 4 °C. The plasma was then re-centrifuged at 16000*g* for 10 min at 4 °C to remove all remaining blood cells. Plasma samples were then aliquoted into 1.2 mL and stored at −80 °C. Circulating cfDNA was extracted from 1.2 mLs of maternal plasma using an automatic magnetic bead extraction and purification system (KingFisher Flex, Thermo Scientific &Co. USA) and using a matched cell free DNA Isolation Kit (Cat. no. R0011, Berry Genomics Corporation, China). Quantitation of cfDNA was performed using the Qubit® 2.0 Fluorometer with Qubit® dsDNA HS Assay Kit (Cat. no. Q32851, Invitrogen &Co. USA). The extracted plasma cfDNA was then subjected to library constructing and massively parallel sequencing using the Illumina NextSeq 500 sequencer (Illumina China, China) as described previously [21]. Sequence data was analyzed and the z-score was calculated. The z-score is a moderate evaluation for standardized chromosomal representation with comparison to the euploid genome. cfDNA levels were obtained from pregnant women who were NIPT negative.

2.3. Diagnostic measurements

Based on the International Association of Diabetic Pregnancy Research Group criteria,75 g oral glucose tolerance test at 24–28 weeks of gestation was used to diagnose GDM [22]. ICP was diagnosed based on abnormal liver function and elevated serum bile acids [23]. Women with normal blood pressure who developed hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) after 20 weeks of gestation with or without obvious proteinuria (> 300 mg/24 h), were respectively diagnosed as PE and PIH [24]. Deliveries prior to 37 gestational weeks were diagnosed as preterm birth (PTB). Based on the Chinese reference curve reported in our previous cohort study, small for gestational age (SGA) or large for gestational age (LGA) referred to neonates whose birth weights were < 10th or > 90th percentiles at gestational age [25]. A baby with birth weight > 4000 g was defined as macrosomia. Low birth weight (LBW) was defined as birth weight < 2500 g.

2.4. Statistical analysis

Demographic characteristics of the study cohort was presented using median (interquartile range, IQR) for continuous variables with skewed distribution and numbers (%) for categorical variables. To compare maternal and neonatal characteristics based on pregnancy complications, non-parametric tests (for non-normally distributed continuous variables) and chi square tests (for categorical variables) were used. Unadjusted and adjusted logistic regression analysis with multivariables were used to determine the association of maternal cfDNA levels at NIPT with pregnancy outcomes. Pregnant women who were of advanced age (≥35 years) had an increased risk of some pregnancy complications. Hence, in our multivariable logistic regression model, the potential effect on the relationship between risk and cfDNA levels due to advanced maternal age was assessed. All statistical analyses were performed using the EmpowerStats software (X&Y solutions inc., USA) and R (version 3.4.2, http://www.R-project.org). Statistical significance denoted $P < 0.05$

3. Results

Based on pregnancy complications and maternal plasma cfDNA levels, demographic characteristics of the mothers and their neonates are presented in Table 1 and Table 2. Of the 831 pregnant women who underwent NIPT at 12 to 22 weeks' of gestation, 111 (13.4%), 39 (4.7%), 52 (6.3%) and 14 (1.7%) were diagnosed with GDM, ICP, PE and PIH, respectively. When women with GDM were compared with women who had non-pregnancy complications (NPC), the median maternal age was higher (35 vs. 33 years, $P < 0.01$). However, no differences in age were observed between women in the NPC group and women in the other groups (ICP/PE/PIH). When compared to women in the NPC group, maternal cfDNA levels at NIPT were significantly higher for women in the ICP and PE groups (median cfDNA 7.07/6.42 vs. 5.99 ng/mL, all $P < 0.01$). There were no significant differences in cfDNA levels at NIPT between women in the NPC and GDM/PIH group (median cfDNA 5.99 vs. 5.95/6.44 ng/mL, $P = 0.198$ and $P = 0.889$,

Table 3
Association of maternal plasma cfDNA with major pregnancy outcomes odds.

Outcomes	N(%)	Unadjusted		Adjusted	
		OR (95CI)	*P* value	OR (95CI)[a]	*P* value
GDM	111 (13.3)	1.05 (0.97–1.13)	0.269	1.02 (0.94–1.11)	0.569
GDM in age < 35 years[b]	54 (11.7)	0.90 (0.78–1.03)	0.118	0.85 (0.73–0.99)[d]*	0.041
GDM in age ≥ 35 years[c]	57 (15.4)	1.17 (1.05–1.30)*	0.004	1.16 (1.04–1.29)[d]*	0.010
ICP	39 (4.7)	1.19 (1.07–1.33)**	0.001	1.20 (1.07–1.34)**	0.001
PE	52 (6.3)	1.16 (1.05–1.28)**	0.004	1.14 (1.02–1.26)*	0.018
PIH	14 (1.7)	0.99 (0.79–1.24)	0.916	0.96 (0.75–1.22)	0.715
PTB	36 (4.3)	1.08 (0.96–1.22)	0.203	1.07 (0.94–1.21)	0.317
SGA	52 (6.3)	0.95 (0.84–1.07)	0.393	0.96 (0.85–1.09)	0.508
LGA	170 (20.5)	0.99 (0.92–1.05)	0.677	0.95 (0.88–1.02)	0.167
LBW	28 (3.4)	1.08 (0.94–1.23)	0.275	1.07 (0.93–1.24)	0.358
Macrosomia	68 (8.2)	1.00 (0.92–1.10)	0.927	0.97 (0.88–1.08)	0.607

Abbreviations: OR, odds ratio; CI, confidence interval; cfDNA, cell free DNA; GDM, gestational diabetes mellitus; ICP, intrahepatic cholestasis of pregnancy; PE, preeclampsia; PIH, pregnancy-induced hypertension; PTB, pre-term birth; SGA/LGA small/large for gestational age; LBW, low birth weight.
Notes:
[a] Adjusted for maternal age, gravidity, parity, gestational age and BMI at NIPT.
[b] Study populations with age < 35 years.
[c] Study populations with age ≥ 35 years.
[d] Adjusted for gravidity, parity, gestational age and BMI at NIPT.
* $P < 0.05$,
** $P < 0.01$.

Table 4
Effect size of maternal cfDNA concentrations at NIPT on GDM in the subgroups by advanced age.

High cfDNA and advanced age	GDM	Unadjusted		Adjusted	
	N (%)	OR (95CI)	*P* value	OR (95CI)[a]	*P* value
cfDNA ≤6.94 ng/mL and < 35 years	46 (14.4)	Reference		Reference	
cfDNA > 6.94 ng/mL and < 35 years	8 (5.7)	0.41 (0.19–0.90)*	0.026	0.38 (0.17–0.83)*	0.016
cfDNA ≤6.94 ng/mL and ≥ 35 years	27 (7.3)	0.83 (0.50–1.39)	0.479	0.7 (0.4–1.3)	0.303
cfDNA > 6.94 ng/mL and ≥ 35 years	30 (21.7)	1.77 (1.0.05–2.97)*	0.031	1.49 (0.85–2.62)	0.163
P value for interaction		< 0.001		< 0.001	

Abbreviations: OR, odds ratio; CI, confidence interval; cfDNA, cell free DNA; NIPT, Non-invasive prenatal test; GDM, gestational diabetes mellitus.
Notes
[a] Adjusted for maternal gravidity, parity, gestational days and BMI at NIPT.
* $P < 0.05$,

respectively). Significant differences were observed for maternal age, BMI at NIPT and the incidence of ICP and PE between women with high cfDNA levels and those with low cfDNA levels (for age: 34 vs. 33 years, $P < 0.05$; for BMI: 22.8 vs. 22.5, $P < 0.05$; for ICP: 7.5% vs. 3.3%, $P < 0.01$; for PE: 8.6% vs. 5.1%, $P < 0.05$) (Table 2). In addition, the incidence of ICP was significantly increased between the maternal cfDNA tertile levels (from 2.6% to 7.5%, $P < 0.05$) (Supplementary Table S1).

The association of maternal plasma cfDNA levels at NIPT with pregnancy complications are presented in Table 3. After adjusting for confounding factors, every ng/mL increase in cfDNA levels at NIPT was associated with an increased risk for ICP (adjusted OR = 1.20, 95% CI: 1.07–1.34, $P < 0.01$) and PE (adjusted OR = 1.14, 95% CI: 1.02–1.26, $P < 0.05$). Plasma cfDNA levels were associated with risk of GDM that was stratified for maternal advanced age (for maternal age ≥ 35 years: adjusted OR = 1.16, 95% CI: 1.04–1.29, for maternal age < 35 years: adjusted OR = 0.85, 95% CI: 0.73–0.99; all $P < 0.05$). However, there was no significant association between cfDNA levels and other pregnancy outcomes (PIH/PTB/SGA/LGA/LBW/macrosomia).

With regards to the risk of GDM, statistically significant interaction between maternal advanced age and high cfDNA levels were observed (*P* value for interaction < 0.001, Table 4). For interaction analysis, the third tertile for cfDNA levels (> 6.94 ng/mL) and maternal age (≥ 35 years) was defined as high and advanced age, respectively. Women with advanced age (≥ 35 years) and high cfDNA levels (> 6.94 ng/mL) had a 1.77–fold higher risk of GDM when compared to women with age (< 35 years) and low cfDNA levels (≤6.94 ng/mL) (OR = 1.77; 95% CI: 1.05–2.97; $P < 0.05$). However, this relationship was not statistically significant after adjusting for confounding factors (adjusted OR = 1.49; 95% CI: 0.85–2.62; $P = 0.163$). The association between maternal plasma cfDNA levels at NIPT and GDM stratified by advanced maternal age is shown in Table 5. Pregnant women ≥35 years and cfDNA levels > 6.94 ng/mL were associated with a higher prevalence of GDM (adjusted OR = 2.04; 95% CI: 1.10–3.79, $P < 0.05$) compared to women with cfDNA ≤6.94 ng/mL. Pregnant women < 35 years and cfDNA levels > 6.94 ng/mL had a 62% lower risk of GDM (adjusted OR = 0.38; 95%CI: 0.17–0.83, $P < 0.05$) compared to pregnant women with cfDNA ≤6.94 ng/mL.

4. Discussion

This is the first retrospective study that associated maternal plasma cfDNA levels at NIPT and subsequent risks of pregnancy complications in a Chinese population. We demonstrated that maternal cfDNA levels at 12–22 weeks of gestation were positively associated with the risk of PE in a Chinese NIPT population, and was consistent with previous publications using other ethnic groups [10,11]. Furthermore, we demonstrated that high maternal cfDNA levels at NIPT was significantly associated with an increased risk of ICP. To the best of our knowledge, this is the first observational study that correlated increased cfDNA levels at NIPT with ICP risk. In addition, we were the first to show that higher cfDNA levels in pregnant women who were ≥35 years had an

Table 5
Associations between maternal plasma cfDNA levels at NIPT and GDM stratified by advanced maternal age.

Plasma cfDNA (ng/mL)	GDM	Unadjusted		Adjusted	
	N(%)	OR (95CI)	*P* value	OR (95CI)[a]	*P* value
Maternal Age < 35 years					
Tertiles					
T1 (< 5.07)	22 (13.8)	Reference		Reference	
T2 (5.07–6.94)	24 (15.0)	1.11 (0.59–2.08)	0.749	0.80 (0.41–1.59)	0.532
T3 (> 6.94)	8 (5.7)	0.43 (0.18–1.01)	0.052	0.34 (0.14–0.83)*	0.018
P value for the trend		0.606		0.018	
Categories					
T1–T2 (≤6.94)	46 (14.4)	Reference		Reference	
T3 (> 6.94)	8 (5.7)	0.41 (0.19–0.90)*	0.026	0.38 (0.17–0.83)*	0.016
Maternal Age < 35 years					
Tertiles					
T1 (< 5.07)	16 (14.2)	Reference		Reference	
T2 (5.07–6.94)	11 (9.7)	0.61 (0.27–1.40)	0.246	0.69 (0.29–1.62)	0.392
T3 (> 6.94)	30 (21.7)	1.69 (0.86–3.33)	0.126	1.71 (0.83–3.53)	0.144
P value for the trend		0.051		0.076	
Categories					
T1–T2 (≤6.94)	27 (7.3)	Reference		Reference	
T3 (> 6.94)	30 (21.7)	2.13 (1.19–3.79)*	0.010	2.04 (1.10–3.79)*	0.024

Abbreviations: OR, odds ratio; CI, confidence interval; cfDNA, cell free DNA; NIPT, Non-invasive prenatal test; GDM, gestational diabetes mellitus; NPC, non–pregnancy complication.
Notes
[a] Adjusted for maternal gravidity, parity, gestational days and BMI at NIPT.
* $P < 0.05$,

increased risk of GDM, however this risk was inversely correlated in pregnant women who were < 35 years old. Maternal age may be an important effect regulator on the association of cfDNA levels with GDM.

Maternal cfDNA contains nearly 95% of maternal derived DNA and 5% of fetal DNA. Maternal DNA is mainly derived from apoptotic hematopoietic cells, while placenta and fetal blood cells are the main source of circulating fetal DNA [26]. Our results demonstrated that in women who subsequently developed PE and ICP, the plasma concentrations of maternal cfDNA were higher compared to NPC pregnant women. These findings may be attributed to the increased apoptosis of trophoblast cells induced by placental ischemia and the decreased clearance of cfDNA in maternal circulation due to the decline of kidney and liver function, which are the possible triggers for PE and ICP [22,27]. Kenna et al. previously demonstrated that the levels of β-cell derived insulin DNA, a component of maternal cfDNA, was significantly lower in women with GDM compared to healthy pregnant women in the 2nd and 3rd trimester of pregnancy. Additionally, Thurik et al. calculated multiples of the median (MoMs) of fetal DNA during early pregnancy based on patient characteristics (BMI, parity, ethnicity and smoking) and reported that fetal DNA MoMs were significantly lower in women who developed GDM compared with healthy pregnant women [29]. The decrease of maternal derived DNA or fetal DNA may lead to a reduction in maternal cfDNA levels. Hence, we could hypothesize that maternal plasma cfDNA levels may decrease in women who develop GDM prior to disease diagnosis as a result of decreased maternal derived DNA and/or fetal DNA. In pregnant women < 35 years old, we observed that increase in early-second cfDNA levels at NIPT was associated with a decreased risk of GDM. It is worth mentioning that the mean or median age of study population in previous two publications were younger than 35 years old [28,29]. Conversely, we observed that higher maternal cfDNA levels contributed to an increased risk of GDM in pregnant women who were ≥35 years old. The mechanism contributing to this paradox is unclear. Possible hypothesis could be age-related alterations in body fat distribution and/or changes in kidney and liver function may contribute to the differences in association of plasma cfDNA levels with risk of GDM in young versus older pregnant women. Maternal advanced age has been found to increase the risk of GDM. Schaefer et al. reported that women older than 35 years had a 3.95–fold higher risk of GDM compared to women aged 16–25 years old (95% CI 2.80–5.58) [30]. Our previous prospective study confirmed that maternal age was an independent risk factor for GDM [31,32]. Characteristics that are associated with aging are changes in body fat distribution and liver and kidney function. Maternal cfDNA levels have been shown to positively correlate with BMI during normal human pregnancy [33]. In addition, significant increase in maternal cfDNA levels were observed with BMI at NIPT in the present study. A previous study suggested that active remodeling of adipose tissue in obese women leads to an increased release of maternal derived cfDNA into the peripheral circulation [34]. In addition, changes in liver and kidney function with age may delay cfDNA clearance [27]. More detailed studies are necessary to elucidate the underlying pathophysiological mechanisms of these age-related differences.

Maternal cfDNA levels in the majority of previously published studies were measured by quantifying specific gene sequence, i.e., GAPDH, β-Globin, HYP2, unmethylated RASSF1A, etc. [10,35,36]. Using different genes to quantify cfDNA levels may contribute to differences in maternal cfDNA levels due to inherent variations between individuals [37]. In this study, maternal cfDNA levels were measured using fluorescence detection methods that were easier to perform and were more economical. During NIPT, a reasonable DNA level determines whether a DNA library could be successfully constructed. In a recent study, Suzumori et al. showed that the percentage of the fetal fraction at NIPT in GDM maternal plasma were not significantly different compared to healthy pregnant women [38]. The fetal fraction, which is the number of fetal cfDNA divided by the total amount of cfDNA, is a key factor to determine the appropriate representation of fetal chromosomes. In the current study, we were unable to obtain specific fetal fractions from sequencing to assess the relationship with pregnancy complications.

Our study had several strengths as well as limitations that should be considered when interpreting our findings. We performed this observational study in our hospital and hence variation in prenatal and obstetric care was minimal. In addition, each maternal blood sample was analyzed in the same laboratory and hence minimized variations. Diagnoses of pregnancy outcomes were confirmed by an investigator who was unaware of the study protocol at the time of data acquisition. We considered any available confounding factors in our statistical analysis. The limitations of our study are as follows; we obtained clinical data without any experimental setting in our retrospective cohort study. Regardless of the number of sample studied, we selected observational participants who received NIPT during the early-second trimester. This limited the generalizability of our findings. Second, despite adjusting for potential confounders in our analysis, we could not rule out other possible factors that could have affected our analysis. Third, we did not analyze the relationship between specific fetal fractions at NIPT and the risk for adverse pregnancy outcomes due to the lack of relevant data. Prospective multi-center studies with larger cohorts are necessary to further investigate maternal cfDNA levels before and during pregnancy to determine its predictive value for pregnancy outcomes.

5. Conclusion

Pregnant women with higher plasma cfDNA levels at NIPT are more likely to develop ICP and PE. Maternal age may be an important effect modifier on the relationship between plasma cfDNA levels and GDM. Maternal cfDNA at NIPT measured in our study had risk assessment

value for ICP, PE and GDM in addition to detecting common chromosomal aneuploidies.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.07.001.

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Disclosure of interests

The author reports no conflicts of interest in this work.

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