Original Research

Circulating lncRNAs NONHSAT054669.2 and ENST00000525337 can be used as early biomarkers of gestational diabetes mellitus

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Impact Statement

Gestational diabetes mellitus (GDM) is a pregnancy complication, seriously affecting the health of mothers and their springs. Fetal development has already been affected by GDM diagnosed at 24–28 weeks of gestation, suggesting the urgent need for screening early diagnostic biomarkers for GDM. We performed lncRNA microarray analysis and detected key lncRNAs expression in different trimesters of pregnancy. Moreover, we analyzed the relationship of key lncRNAs expression with blood glucose in oral glucose tolerance test (OGTT) of GDM pregnant women during the second trimester and evaluated the performance of key lncRNAs for diagnosing GDM during different trimesters. Our study showed that the expression of lncRNAs changed dynamically in different trimesters, providing new insights into the early diagnosis of GDM.

Abstract

Early diagnosis can help prevent and reduce the adverse effects of gestational diabetes mellitus (GDM). This study intended to investigate key circulating long non-coding RNAs (lncRNAs) as novel biomarkers for diagnosis of GDM at the early stages. First, lncRNA microarray analysis was conducted for plasma samples of GDM women before delivery and 48 h after delivery. The expression of differentially expressed lncRNAs in clinical samples at different trimesters was randomly validated by quantitative polymerase chain reaction (PCR). Moreover, the correlation between lncRNA expression and oral glucose tolerance test (OGTT) level in GDM women during the second trimester was analyzed, followed by evaluating the diagnostic value of key lncRNAs during different trimesters using receiver operating characteristic (ROC) curve. Higher NONHSAT054669.2 expression and lower ENST00000525337 expression were revealed in GDM women before delivery relative to 48 h after delivery ($P < 0.05$). The expression of NONHSAT054669.2 and ENST00000525337 in GDM women during the first and second trimesters was dramatically higher than pregnant women ($P < 0.05$) with normal glucose tolerance (NGT). During the second trimester, NONHSAT054669.2 expression was positively related to OGTT level at 1 h ($r = 0.41455$, $P < 0.001$). Furthermore, ROC curve analysis revealed that ENST00000525337 alone, NONHSAT054669.2 alone, and their combination had high diagnostic value for GDM during the first (area under the ROC curve (AUC) = 0.979, 0.956, and 0.984, respectively) and second (AUC = 0.829, 0.809, and 0.838, respectively) trimesters (all $P < 0.001$). The plasma level of NONHSAT054669.2 and ENST00000525337 may be applied as novel diagnostic biomarkers for early diagnosis of GDM.

Keywords: Early pregnancy, gestational diabetes mellitus, circulating RNA, lncRNA, biomarkers, diabetes

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Introduction

Gestational diabetes mellitus (GDM) affects about 7% of all pregnancies and its prevalence is on the rise worldwide.[1,2] It has significant short- and long-term adverse effects for both the mother and her offspring.[3–7] The currently available gold standard for GDM diagnosis is 75 g oral glucose tolerance test (OGTT) at 24–28 weeks of gestation;[8] however, in older and/or obese women, GDM diagnosed at this period have already affected fetal abdominal obesity.[9] Therefore, early diagnosis and timely treatment of GDM have great significance to prevent or considerably reduce the risk of adverse consequences on patients and their children.

A growing number of studies are devoted to discover the promising biomarkers for early diagnosis of GDM. For instance, circulating levels of nesfatin-1 and vaspin

are decreased in GDM pregnant women and may be used for prediction and early diagnosis of GDM.[10] A multivariate classification model combined by several first trimester pregnancy blood-borne biomarkers, including cholesterol, insulin, triglycerides, homeostatic model assessment, tissue plasminogen activator, and low-density lipoprotein, has a high clinical utility for diagnosis of GDM.[11] Increased levels of plasma metabolites like 17(S)-HDoHE and sebacic acid in GDM pregnant women may be applied to early prediction of GDM.[12] Plasma miR-17-5p, miR-16-5p, and miR-20a-5p exhibit high value in distinguishing GDM women and non-GDM women and may be promising diagnostic biomarkers in GDM.[13] However, the utility of some biomarkers is limited by low accuracy or affected by sample type or analysis methods,[14,15] emphasizing the requirement for additional early diagnostic biomarkers for GDM.

Long non-coding RNAs (lncRNAs) have been reported to participate in various physiological and pathological processes.[12–14] Dysregulation of key circulating or placenta-related lncRNAs can affect insulin resistance and β-cell dysfunction in GDM development, and may lead to changes in target gene expression in the offspring and consequently result in the development of GDM-related complications like cardiovascular and metabolic diseases.[16] Recently, circulating RNAs in the plasma or serum have become an emerging field of noninvasive diagnostic applications.[17] Several lncRNAs in the plasma have been used as potential biomarkers for various diseases such as cancer,[18] coronary artery disease,[19] T2DM,[20] and GDM.[21] Nevertheless, the lncRNAs in the plasma that can be used for diagnosis of GDM at early stages are largely unknown.

Herein, we performed lncRNA microarray analysis for plasma samples of GDM pregnant women before delivery and 48 h after delivery to identify key lncRNAs associated with GDM, followed by detection of key lncRNA expression in clinical samples of different trimesters of pregnancy by quantitative polymerase chain reaction (qPCR). Moreover, we analyzed the correlation of key lncRNA expression with blood glucose in OGTT in GDM pregnant women during the second trimester and evaluated the value of key lncRNAs for diagnosing GDM during different trimesters. Our findings will lay the foundation for diagnosis of GDM at the early stages.

Materials and methods

Patients and sample collection

This retrospective study was approved by the Ethics Committee of the Second Hospital of Shandong University, and all participants were informed consent for research during the specimen collection process.

From December 2016 to December 2018, pregnant women were collected from the Second Hospital of Shandong University. According to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommendations,[22] pregnant women underwent OGTT between 24 and 28 weeks of gestation. GDM diagnosis referred to fasting plasma glucose ⩾ 5.1 mmol/L, or post 75 g glucose level at 1 h ⩾ 10.0 mmol/L or at 2 h ⩾ 8.5 mmol/L. Women with pre-pregnancy diabetes, pregnancy-induced hypertension, threatened premature birth, chronic hypertension, multiple pregnancy, and premature birth, and fetal growth restriction were excluded.

Pregnant women were divided into four groups: third-trimester (36–41 weeks) GDM group (39 GDM pregnant women before delivery and 48 h after delivery), third-trimester normal glucose tolerance (NGT) group (37 NGT pregnant women before delivery and 48 h after delivery), second-trimester group (24–28 weeks) (56 GDM and 58 NGT pregnant women), and first-trimester group (12–14 weeks) (27 GDM women and 45 NGT women). In the first trimester group, the blood samples of pregnant women were collected for the presence of fetal Down's syndrome, and then GDM or NGT was diagnosed according to OGTT results in the second trimester. GDM and NGT pregnant women in all cohorts were matched by age.

Fasting peripheral venous blood (5 mL) was collected and centrifuged at 3000 rpm for 10 min. The plasma was then collected immediately and stored at −80°C.

LncRNA microarray analysis

Total RNA extraction from plasma of three GDM women before delivery and 48 h after delivery was conducted using RNeasy Total RNA Isolation Kit (Qiagen, GmBH, Germany). Total RNA was then purified using an RNeasy Mini Kit (Qiagen) and its concentration was determined on an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The cRNA synthesis was conducted, followed by labeling with Sino Human ceRNA array V3.0 (Sinamics Corporation, China). This lncRNA array contained 591,614 ncRNA and 25,353 mRNA probes. After hybridization, we scanned the array using the Agilent Microarray Scanner (Agilent Technologies) and extracted raw data with Feature Extraction software 10.7 (Agilent Technologies). Raw data were then quantile normalized by limma package in R.

Analysis of differentially expressed lncRNAs and their function

The differentially expressed lncRNAs in GDM women between before delivery and 48 h after delivery were obtained with the cutoff value of fold change > 2 and adjusted P value < 0.05.

To better understand the function of differentially expressed lncRNAs, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were done using Fisher's exact test by clusterProfiler package in R. Significant GO and pathway terms were selected with P value < 0.05.

qPCR

Total RNA extraction from plasma of patients was using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was then purified using RNeasy Mini Kit (Qiagen) and quantified using Nanodrop ND 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription to cDNA was carried out with RevertAid™ First Strand cDNA Synthesis Kit (Fermentas; Thermo Fisher Scientific), followed by analysis of lncRNA expression using qPCR with the Maxima SYBR Green qPCR Master Mix (Thermo Scientific) on the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). qPCR reaction conditions

Table 1. The primer sequences used in this study.

lncRNAs	Primer sequences (5′-3′)	
	Forward	Reverse
NONHSAT024258.2	CAACGAGTCCAACTTCAGTG	CCTTCAGGTCTTTCTCACAC
NONHSAT117910.2	ATTCCTCATCATGGGCATCG	GTACTGTTCTTACTTGAAGCCTG
NONHSAT114000.2	TGTTATGTGTTCTCCGTTGAC	CAATTCCCTTGACCATATCTCTG
NONHSAT117394.2	TGTTATGTGTAGGAGGAAGAG	TCATGTGCTTCACAACAGAG
NONHSAT054669.2	ATGATGGCACAGGAAGGGAATG	GTGGATTTGCTGGCAGGTTTC
NONHSAT137452.2	AACCGTGTTACCATACTCTGTGA	CCTTGATTTGCCTTCCTCTAA
NONHSAT092527.2	GAGTTCACCAGTGTATTAACTACC	CTAAGCAGTTGGTGACACAG
ENST00000525337	GACTGGCGAGCCGAAGATTTA	CTTTATGGGAGCCGATGAGGT
ENST00000567396	GCCTGTTGAGAACTTGTGGAT	ATATGTCAGCCCTCAGTATGG
ENST00000613256	CAGCAAGAGGTTGGTCTGAAT	AAAGGGGCAAGGGGAGAAATA
NONHSAT091500.2	GGGGTCTCGCTAAGAAGGAGG	CCATAGGCAGTTCGCAACATG
NONHSAT221603.1	GTCTTGCTGGATAATCAAATGC	GTCTTGCTGGATAATCAAATGC
NONHSAT126573.2	TGAAGTAGGAGATAGCGATGAC	TGTGTCCTCTATACCACCCT
NONHSAT176455.1	CCATTGACACCTACCAGGAG	CTAGATTGGATTTCTGTTGCGT

were denaturation at 95°C for 10 min and then 40 cycles at 95°C for 15 s and 60°C for 60 s. The primer sequences were shown in Table 1. The relative lncRNAs expression was determined using the $2^{-\Delta\Delta Ct}$ method. GAPDH was applied as the internal control.

Statistical analysis

Statistical analysis was completed using SPSS 22.0 software (IBM Co., Armonk, NY, USA). Data normality was evaluated by the Shapiro–Wilk test. If normally distributed, data were expressed as mean ± standard deviation (SD) and their differences between two groups were compared by paired *t*-test. Otherwise, data were represented by median and quartile intervals, and the Wilcoxon test was applied to analyze the data. Categorical variables were displayed as numbers (%) and compared using Pearson's chi-square test. The correlation between lncRNA expression and blood glucose in OGTT in GDM pregnant women was analyzed using Pearson correlation analysis. The diagnostic value of key lncRNAs for GDM during three trimesters was evaluated using receiver operating characteristic (ROC) curve analysis. At the same time, we also conducted a comparative analysis of the differences between the two groups. Since the data did not obey the normal distribution, we used a nonparametric test for comparison, considering the differences in the median and the differences in the differential confidence intervals. $P < 0.05$ was considered statistically significant.

Results

Identification of differentially expressed lncRNAs in GDM pregnant women between before delivery and 48 h after delivery

Since blood glucose and other metabolic indicators of GDM pregnant women were completely normal at 48 h postpartum, we identified differentially expressed lncRNAs in three GDM women between before delivery and 48 h after delivery by lncRNA microarray analysis, aiming to screen GDM-related lncRNAs. The clinical characteristics of these three patients are demonstrated in Supplementary Table 1. As a result, 1057, 13,950, and 1982 differentially expressed lncRNAs between before delivery and 48 h after delivery were respectively obtained from three GDM pregnant women (Figure 1(A)). Venn diagrams illustrated that 91 (41 upregulated and 50 downregulated) common differentially expressed lncRNAs were obtained from three GDM pregnant women (Figure 1(B)). Moreover, these differentially expressed lncRNAs were remarkably enriched in multiple GO terms such as ATP biosynthetic process, integral component of Golgi membrane, and cytochrome-c oxidase activity (Figure 1(C)), and KEGG pathways like NOD-like receptor signaling pathway, oxidative phosphorylation, lysosome, and p53 signaling pathway (Figure 1(D)).

To verify the reliability of lncRNA microarray analysis, 14 differentially expressed lncRNAs in the three GDM pregnant women used for lncRNA microarray analysis were randomly selected and their expression was verified by qPCR. We found that the expression trend of 12 differentially expressed lncRNAs in the three GDM pregnant women was in line with the results of lncRNA microarray analysis (Figure 1(E)).

Analysis of key lncRNAs associated with GDM

To further identify key lncRNAs associated with GDM, seven differentially expressed lncRNAs with the greatest difference were selected for further verification in more plasma samples of GDM ($n = 39$) and NGT ($n = 37$) pregnant women before delivery and 48 h after delivery. The baseline characteristics of pregnant women during the third trimester are shown in Supplementary Table 2. Notably, we found that the expression of NONHSAT054669.2 in GDM pregnant women before delivery was significantly higher than that at 48 h after delivery, while the ENST00000525337 expression was obviously lower in GDM pregnant women before delivery in comparison with that at 48 h after delivery ($P < 0.05$, Figure 2(A); the differential confidence intervals of lncRNAs with $P < 0.05$ in Supplementary Table 4), which were in line with the results of lncRNA microarray analysis. However, the expression levels of NONHSAT054669.2 and ENST00000525337 in NGT pregnant women were not different between before delivery and 48 h after delivery (Figure 2(B)).

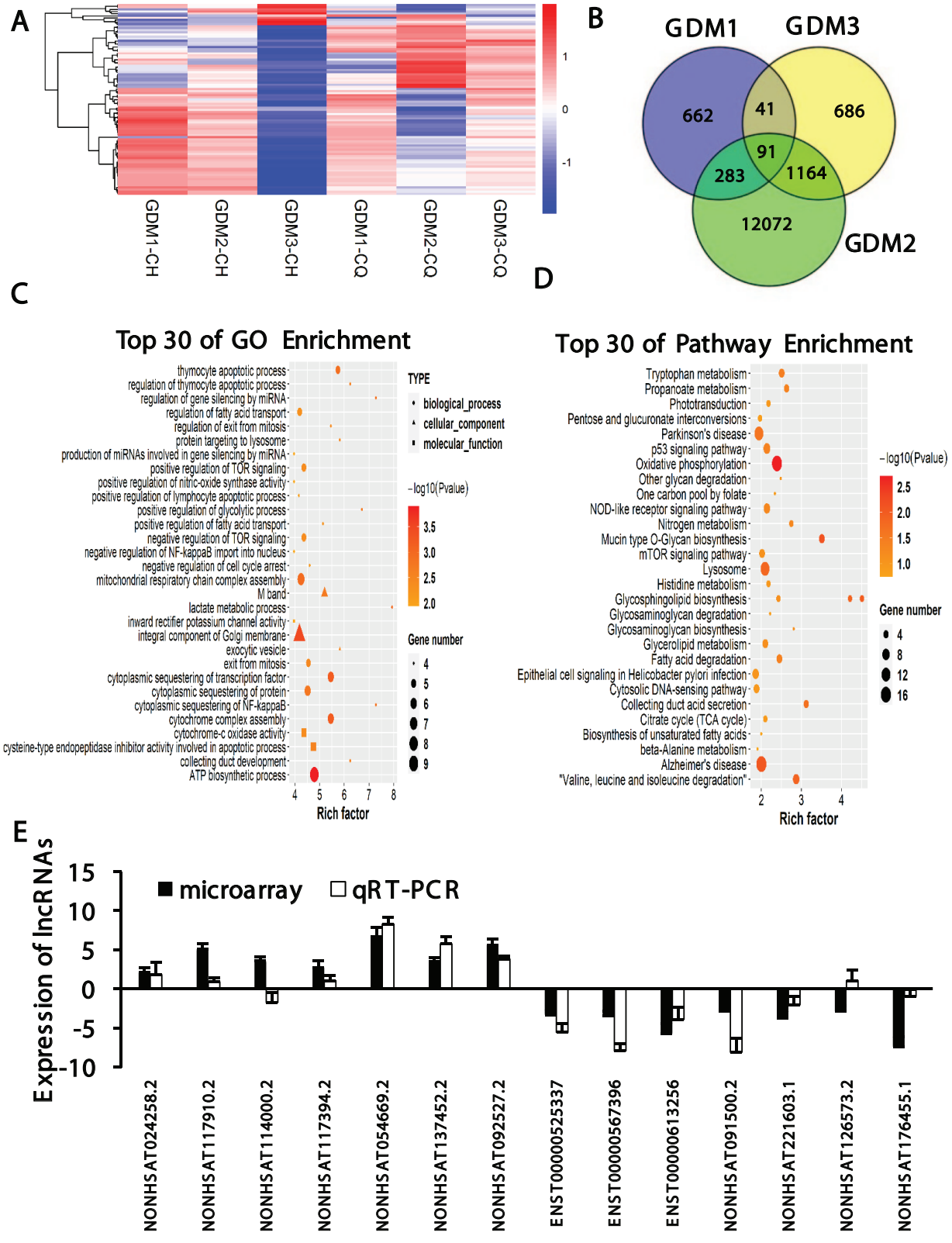

Figure 1. Differentially expressed lncRNAs in GDM pregnant women between before delivery (GDM-CQ) and 48 h after delivery (GDM-CH) and their functional analysis. (A) Heatmap of differentially expressed lncRNAs in three GDM pregnant women. (B) Venn diagrams illustrated that common differentially expressed lncRNAs in three GDM pregnant women. (C) The top 30 Gene Ontology (GO) terms enriched by differentially expressed lncRNAs. (D) The top 30 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched by differentially expressed lncRNAs. (E) LncRNA microarray analysis and qPCR showed the expression of randomly selected 14 differentially expressed lncRNAs in the three GDM pregnant women.

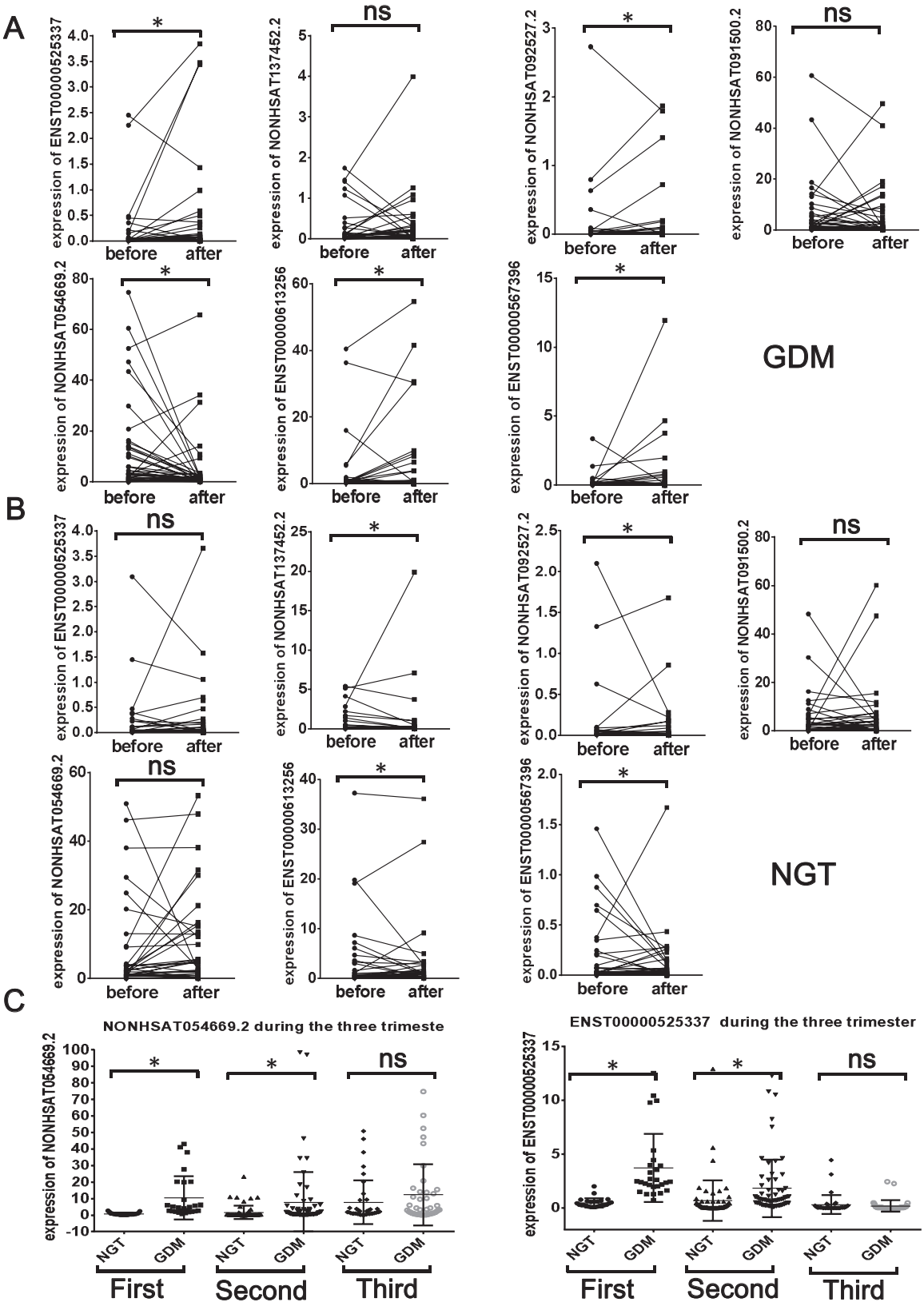

Figure 2. qPCR showed the expression of key lncRNAs associated with GDM. (A) The expression of seven differentially expressed lncRNAs with the most significant difference in more plasma samples of GDM pregnant women before delivery and 48 h after delivery; (B) the expression of seven differentially expressed lncRNAs with the most significant difference in more plasma samples of normal glucose tolerance (NGT) pregnant women before delivery and 48 h after delivery; and (C) the expression of NONHSAT054669.2 and ENST00000525337 in the GDM and NGT pregnant women during the first trimester (12–14 weeks), second trimester (24–28 weeks), and third trimester (36–41 weeks).

We further determined the expression of NONHSAT054669.2 and ENST00000525337 in the GDM and NGT pregnant women during the first (12–14 weeks), second (24–28 weeks), and third (36–41 weeks) trimesters (Figure 2(C)). It was found that although the expression of NONHSAT054669.2 increased gradually with pregnancy in GDM and NGT pregnant women, NONHSAT054669.2 expression in GDM pregnant women during the first and second trimesters was all visibly higher than that in NGT pregnant women ($P < 0.05$, the differential confidence intervals of NONHSAT054669.2 during the three trimesters in Supplementary Table 5). In addition, the expression of ENST00000525337 in GDM pregnant women decreased gradually with the increase of pregnancy, but this phenomenon was not observed in NGT pregnant women. Moreover, with the increase of pregnancy, the difference of ENST00000525337 expression between GDM and NGT pregnant women became less and less obvious. Higher ENST00000525337 expression was observed in GDM pregnant women in comparison with NGT pregnant women during the first and second trimesters ($P < 0.05$, the differential confidence intervals of ENST00000525337 during the three trimesters in Supplementary Table 6), but there was no significant difference between GDM and NGT pregnant women during the third trimester. The baseline characteristics of pregnant women during three trimesters are shown in Supplementary Tables 2 and 3.

Correlation analysis of the expression of NONHSAT054669.2 and ENST00000525337 during the second trimester with blood glucose in OGTT

We further assessed the correlation of NONHSAT054669.2 and ENST00000525337 expression during the second trimester with blood glucose in OGTT. We found that NONHSAT054669.2 expression was positively related to blood glucose at 1 h in GDM pregnant women ($r = 0.41455$, $P < 0.001$, Figure 3(A)). The ENST00000525337 expression was negatively correlated with blood glucose at 0 h, but statistical significance was not obvious ($r = -0.17946$, $P = 0.1628$, Figure 3(B)). These data suggested that the two lncRNAs might be involved in GDM pathogenesis.

Analysis of the diagnostic value of NONHSAT054669.2 and ENST00000525337 for GDM during different trimesters

We further evaluated the diagnostic value of NONHSAT054669.2 and ENST00000525337 in GDM pregnant women at the first, second, and third trimesters by ROC curve analysis (Table 2 and Figure 4). The results showed that at the first trimester, the area under the ROC curve (AUC) value of ENST00000525337, NONHSAT054669.2, and their combination was 0.979, 0.956, and 0.984, respectively; and at the second trimester, the AUC value was 0.829, 0.809, and 0.838, respectively. However, at the third trimester, the AUC value was 0.553, 0.596, and 0.636, respectively. Overall, ENST00000525337 alone, NONHSAT054669.2 alone, and their combination had the ability to distinguish GDM and NGT pregnant women at the early stages.

Discussion

GDM seriously threatens maternal and infant health. A previous study has shown that GDM patients diagnosed at early stages have much better pregnancy outcomes than those diagnosed at advanced stages because some pathological changes such as chorangiosis and villous fibrinoid necrosis cannot be completely reversed in GDM patients with advanced stage.[23] Most pregnant women with GDM are definitively diagnosed in the early trimester of pregnancy; however, the diagnosis accuracy depends on the individual standards, the environment difference, and the diverse strategies.[24] Furthermore, there is no unified strategy for GDM diagnosis worldwide. Recently, circulating biomarkers have been developed for diagnosis of multiple clinical disorders, including GDM,[25] and our previous study found that plasma exosomal cirRNA could be used for early detection of GDM.[26] To extend the time window for early diagnosis of GDM, more investigations are made to find key circulating lncRNAs as novel biomarkers with diagnostic and therapeutic effects in GDM.

Over the past few decades, lncRNAs are one of the hottest fields of research. Benefiting from the development of microarray analysis and bioinformatics methods, lncRNAs have been identified as critical regulators of human diseases, including GDM.[27] Li *et al*.[28] demonstrated that lncRNA RPL13p5 played a key role in promoting insulin resistance in patients with GDM. LncRNA MEG3 is revealed to be upregulated in GDM and contribute to GDM development by regulating human chorionic trophoblast cell physiology.[29] In addition, an lncRNA microarray analysis has identified 1098 differentially expressed lncRNAs in GDM patients, which may play a significant role in insulin resistance.[30] Our results revealed higher expression of NONHSAT054669.2 and lower ENST00000525337 expression in plasma of GDM patients before delivery in comparison with 48 h after delivery. Moreover, the NONHSAT054669.2 and ENST00000525337 expression levels in GDM patients were distinctly higher than those in NGT women. These data imply that circulating NONHSAT054669.2 and ENST00000525337 may be involved in GDM development. Furthermore, differentially expressed lncRNAs were found to be enriched in multiple metabolism-related functions and pathways like ATP biosynthetic process and oxidative phosphorylation. Furthermore, our data indicated that NONHSAT054669.2 expression was positively related to blood glucose at 1 h in GDM pregnant women during the second trimester. It can thus be speculated that NONHSAT054669.2 might be a key regulator to control blood glucose level in GDM patients.

Disease-associated lncRNAs are reported to be detectable in blood, urine, sputum, and other biological fluids of patients.[31,32] Unlike most protein biomarkers, lncRNAs are stable in blood circulation[33] and play a crucial role in the early diagnosis of diverse diseases.[34,35] LncRNA MALAT1 is found to be increasingly expressed in patients with GDM and has a diagnosis value with the AUC of 0.654.[36] LncRNA HOTAIR is upregulated in GDM pregnant women and has high diagnostic value for GDM (AUC = 0.906).[37] Zhang *et al*.[21] demonstrated that plasma lncRNA MEG8 level could be utilized for selecting patients with high risk

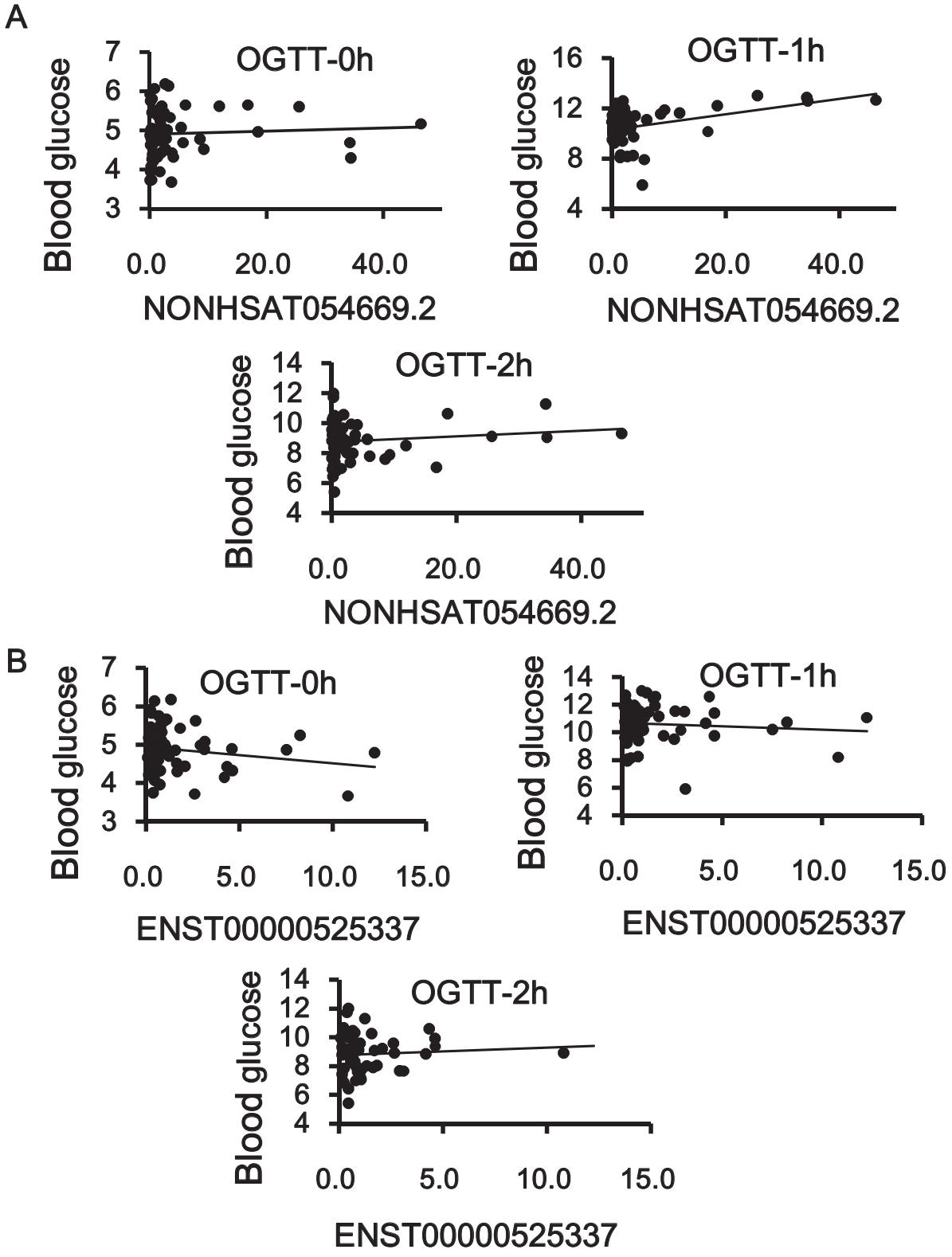

Figure 3. Correlation analysis of the expression of NONHSAT054669.2 and ENST00000525337 with blood glucose in OGTT during the second trimester. (A) Correlation between NONHSAT054669.2 expression and blood glucose in OGTT and (B) correlation between ENST00000525337 expression and blood glucose in OGTT.

of GDM. In addition, Lu *et al.*[38] demonstrated that circulating XLOC_014172 and RP11-230G5.2 combined had a high diagnostic ability for macrosomia in GDM patients with the AUC of 0.955. However, those studies did not analyze the expression and diagnostic value of lncRNA in different stages of pregnancy. In our study (Figure 5), we studied the expression of lncRNA in GDM pregnant women before/after delivery and in different trimesters, indicating that the

Table 2. ROC analysis analyzed the diagnostic significance of ENST00000525337, NONHSAT054669.2 and their combination in GDM patients.

Pregnancy	Variables	AUC	SE	95% CI	*Z* statistic	*P* value
First trimester	ENST00000525337	0.979	0.015	0.895–0.999	31.884	<0.001
	NONHSAT054669.2	0.956	0.0251	0.859–0.993	18.125	<0.001
	Combination	0.984	0.0134	0.902–1.000	36.076	<0.001
Second trimester	ENST00000525337	0.829	0.0395	0.748–0.892	8.324	<0.001
	NONHSAT054669.2	0.809	0.0432	0.725–0.876	7.135	<0.001
	Combination	0.838	0.0384	0.757–0.900	8.784	<0.001
Third trimester	ENST00000525337	0.553	0.067	0.435–0.667	0.791	0.4289
	NONHSAT054669.2	0.596	0.067	0.447–0.708	1.441	0.1496
	Combination	0.636	0.066	0.516–0.745	2.046	0.0407

ROC: receiver operating characteristic; GDM: gestational diabetes mellitus; AUC: area under the ROC curve; SE: standard error; CI: confidence interval.

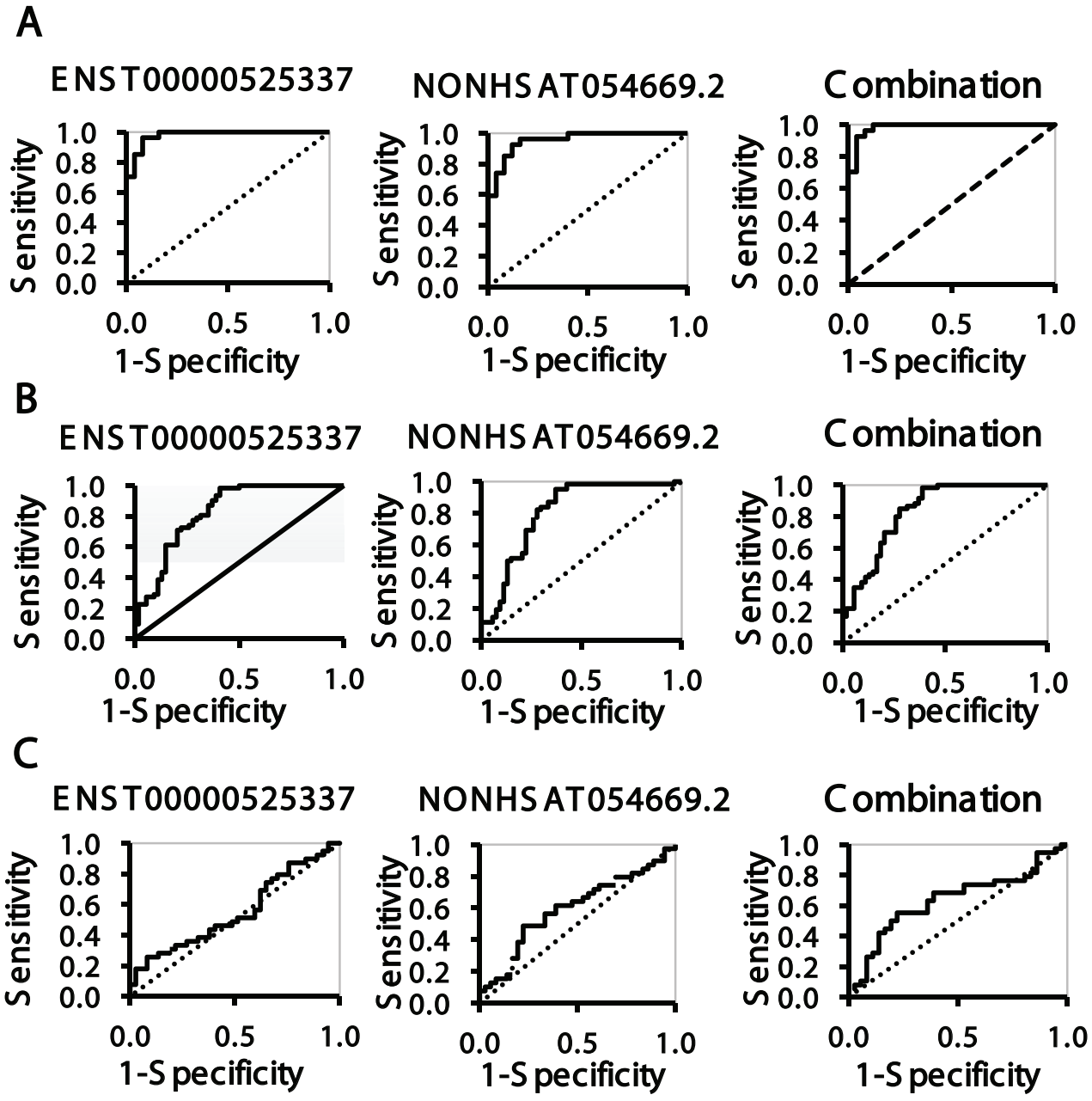

Figure 4. Analysis of the diagnostic value of NONHSAT054669.2 and ENST00000525337 for GDM during the first, second, and third trimesters. (A) In GDM pregnant women at the first trimester, the AUC value of ENST00000525337, NONHSAT054669.2, and their combination was 0.979, 0.956, and 0.984, respectively. (B) At the second trimester, the AUC value was 0.829, 0.809, and 0.838, respectively. (C) At the third trimester, the AUC value was 0.553, 0.596, and 0.636, respectively.

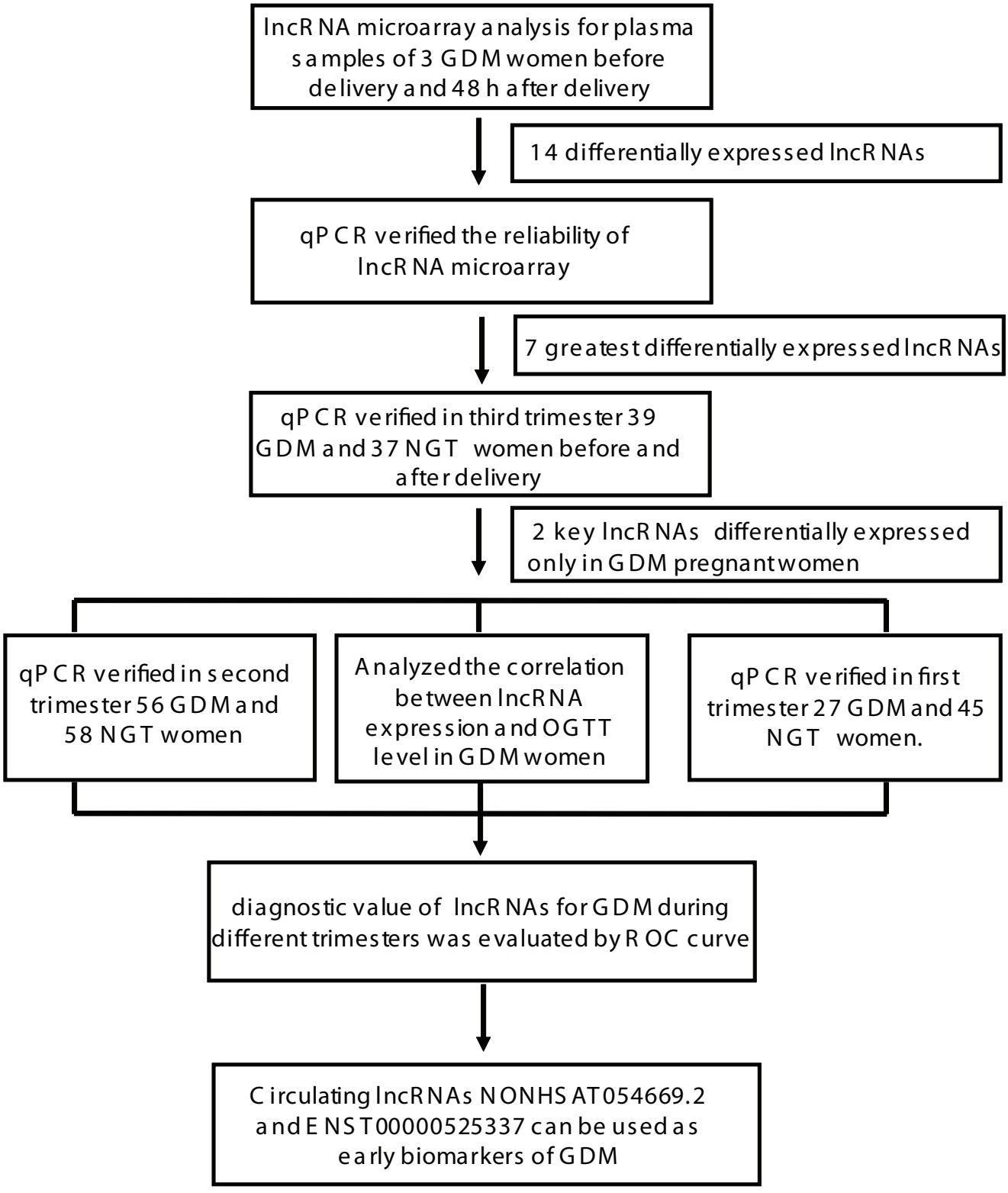

Figure 5. The overall study design of the analysis procedure.

expression of lncRNA was dynamically changing. And the AUC values of ENST00000525337, NONHSAT054669.2, and their combination for GDM diagnosis during the first trimester were 0.979, 0.956, and 0.984, respectively. The AUC values of pregnant women diagnosed with GDM in the second trimester were 0.829, 0.809, and 0.838, and 0.553, 0.596, and 0.636 in the third trimester. Taken together, our findings for the first time hinted that plasma expression levels of NONHSAT054669.2 and ENST00000525337 can be used to predict the risk of GDM in the early stage.

There are some limitations of our study. The functional characterization of NONHSAT054669.2 and

ENST00000525337 in GDM was lacking. The role and mechanism of NONHSAT054669.2 and ENST00000525337 in GDM should be further explored. Moreover, although different biomarkers such as circulating RNAs, single-nucleotide polymorphisms, and DNA methylation were developed for GDM diagnosis, they are frequently affected by gestational age, sample size and type, and detection method.[39] The sample size for ROC analysis of the diagnostic value of NONHSAT054669.2 and ENST00000525337 was small, which may affect the diagnostic test accuracy. The predictive value of the two lncRNAs for GDM should be tested in more populations with different gestational ages and sample sizes.

In conclusion, our findings reveal that monitoring of the plasma NONHSAT054669.2 and ENST00000525337 during pregnancy may be used for predicting the risk of GDM at the early stages, which may provide evidence for finding novel diagnostic biomarkers in clinical application.

AUTHORS' CONTRIBUTIONS

LncRNAs were extracted and verified by WJ. Clinical specimens were collected by GHC. MRX and SYL collected clinical data, which were statistically analyzed by XBS and FFL. Finally, LNW and YW designed the overall research and wrote the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Second Hospital of Shandong University (KYLL-2021(KJ)P-0175).

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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