Maternal serum Lamin A is a potential biomarker that can predict adverse pregnancy outcomes

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Summary

Background Maternal serum Lamin A (LMNA) was reported to have potential diagnostic value in the prenatal diagnosis of congenital heart disease (CHD). In this study, we aimed to further assess the prognostic value of maternal serum LMNA in predicting adverse pregnancy outcomes.

Methods A prospective screening study was performed on singleton pregnancies at 15–18 weeks of gestation. After a routine test for alpha fetoprotein (AFP), chorionic gonadotropin (hCG), and unconjugated estriol (uE3), serum LMNA levels were measured. Serum LMNA levels were then converted into multiples of the median (MoM). The median MoM values for adverse pregnancy outcomes were compared with those in normal pregnancies. For diseases with differential LMNA expression in the prospective study, another case-control cohort was recruited. The diagnostic value of LMNA in these diseases was further evaluated.

Findings Between January 1, 2017 and June 30, 2018, a total of 2906 singleton pregnancies were recruited. Of the 2,906 cases, 2711 had data available for analysis. Congenital structural abnormalities, chromosomal abnormalities, and obstetric complications were observed in 152 (5·6%), 15 (0·6%), and 278 (10·3%) patients, respectively. LMNA was downregulated in pregnancies with fetal CHD, fetal neural tube defects (NTD), and preeclampsia (PE). The case-control study cohort included 256 CHD, 60 NTD, 67 PE, and 400 normal pregnancies. The areas under the curve for the prenatal diagnoses of CHD, NTD, and PE were 0·875, 0·871, and 0·816, respectively.

Interpretation Maternal serum LMNA was found to be a potential biomarker for the prenatal diagnosis of fetal CHD, NTD, and PE.

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Keywords: LMNA; Serum biomarker; Congenital heart defects; Neural tube defects; Preeclampsia

Introduction

The maternal serum α-fetoprotein (AFP) test was first introduced into clinical practice to screen for fetal neural tube defects (NTD) in the 1970s. Since then, various other methods for prenatal screening tests have been developed,[1] but screening tests using pregnancy-related biomarkers in the maternal peripheral blood have been recognized as one of the best methods for prenatal diagnosis,[2] as they help avoid invasive tests, such as amniocentesis and cordocentesis. Moreover, these tests can be performed even in primary hospitals, which can then aid in deciding whether a patient needs a referral for further prenatal evaluation.

As early as 1996, the United States Agency for Healthcare Research and Quality recommended second-trimester maternal serum test screening for Down syndrome and NTDs. These tests include screening for AFP, chorionic gonadotropin (hCG), unconjugated estriol (uE3), and pregnancy-associated plasma protein A (PAPP-A).[3] Triple (AFP, hCG, uE3) or quadruple (AFP, hCG, uE3, PAPP-A) screening methods are still widely used in clinical practice; however, serum screening for other birth defects remains limited. For instance,

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Research in context

Evidence before this study

Congenital heart defects (CHDs) are the most common type of major birth defects. Currently, there are no identified biomarkers used in clinical practice to prenatally detect CHD. In our previous study, however, we reported the potential diagnostic value of maternal serum Lamin A (LMNA) measurements, combined with proteomics and immune-technology, for the prenatal detection of CHD. However, whether LMNA is a CHD-specific biomarker, as well as the best detection time and cut-off values for its application, needs to be further studied.

Added value of this study

Here, we conducted a prospective study on women participating in the second-trimester serum screening test to further explore the value of the LMNA test as a possible biomarker for CHD and other adverse pregnancy outcomes. We discovered that the downregulation of LMNA in maternal serum could not only predict CHD but also NTD and PE antenatally, which was confirmed by another case-control cohort. Additionally, there were significant differences between adverse pregnancies (CHD, NTD, and PE) and normal pregnancies at each gestational age in our study.

Implications of all the available evidence

Maternal serum LMNA was found to be a potential biomarker for the prenatal diagnosis of CHD, NTD, and PE. The LMNA test could be added to the battery of laboratory screening tests conducted in the early second-trimester of pregnancy after other large-scale clinical validation studies have been carried out.

there are currently no biomarkers for the prenatal diagnosis of congenital heart disease (CHD), despite being one of the most common congenital abnormalities. In addition, prenatal screening has been broadened to include screening for pregnancy complications such as pre-eclampsia (PE) and fetal growth restriction (FGR). In recent years, with the development and application of proteomics and other omics to predict adverse pregnancy outcomes, several new prenatal candidate biomarkers have been proposed[4–8]; however, only a few have been applied clinically. In addition, most of these markers were proposed based on case-control studies that explored only a limited and specific set of diseases.

In our previous study,[9] we reported the potential diagnostic value of maternal serum Lamin A (LMNA), combined with proteomics and immune-technology, in the prenatal detection of CHD in pregnant women from 22–26 weeks of gestation; however, several aspects need to be explored further before LMNA can be clinically used. For instance, it must be determined whether LMNA is CHD-specific. In addition, the best gestational age for screening and the best cut-off value need to be determined. If maternal serum LMNA levels can be used to screen for CHD at an earlier stage, the LMNA test may have the potential to be added to the battery of laboratory screening tests conducted in the early second-trimester of pregnancy. Therefore, based on the results of our previous studies, this study aimed to conduct a prospective study in women participating in second-trimester serum screening tests to further explore the value of the LMNA test as a possible biomarker for screening CHD and other adverse pregnancy outcomes. In addition, we conducted another case-control study, involving diseases under which LMNA is differentially expressed, to compensate for the small sample size of certain diseases in the original prospective study.

Methods

Study population

Prospective cohort. Data for the prospective study were obtained from 2906 women attending the routine screening test for Down syndrome at 15–18 weeks of gestation at Shengjing Hospital, China Medical University between January 2017 and June 2018. Detailed obstetric and medical histories were recorded, and maternal weight, height, and arterial blood pressure measurements were obtained for all participants. All pregnant women underwent a PE risk assessment during the first trimester (according to the FMF algorithm using maternal characteristics, medical history, and biophysical measurements) (http://fetalmedicine.org/research/assess/preeclampsia). High risk is classified at a risk value of ≥1/100.[10] Furthermore, maternal blood was collected, and the serum was immediately harvested from all participants. In brief, 5mL venous blood samples were collected in separating gel vacuum tubes, and samples were centrifuged at 3000 rpm at 4 °C for 15 min. After conducting routine tests for AFP, hCG, and uE3, additional serum was stored at the specimen bank of Shengjing Birth Cohort until June 2018 to complete the recruitment period.

Case-control study cohort. As the prevalence of several adverse pregnancy outcomes was not high enough to obtain a sufficient sample size for the prospective study, we performed another case-control study using the serum samples obtained from women diagnosed with adverse pregnancy outcomes (CHD, NTD, and PE). The samples in the case-control study were completely independent of those in the prospective study. The diagnoses of CHD and NTD were based on prenatal ultrasound and confirmed postnatally by postnatal imaging examinations, and/or surgery, and/or autopsy.

PE was defined in accordance with the American College of Obstetricians and Gynecologists (ACOG) guidelines, as systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg on at least two occasions four hours apart, developing from 20 weeks of gestation onwards in previously normotensive women and proteinuria ≥300 mg in a 24 hour urine specimen. In absence of proteinuria, it was considered the new onset of hypertension with new onset of any of the following: (1) Trombocitopenia: Platelet count <100,000/μL; (2) Renal insufficiency, characterized by a serum creatinine concentration greater than 1.1 g/dL or doubling the serum creatinine concentration in absence of other renal diseases; (3) Impared liver function: elevated concentrations of liver transaminases to twice normal concentration; (4) Pulmonary edema; (5) Cerebral or visual symptoms.[11] Early- and late-onset PE were defined as PE requiring delivery before or after 34 weeks of gestation, respectively. Pregnancies with other malformations or other complications were not included in the study. In addition, we randomly selected 400 healthy women carrying normal fetuses at the corresponding gestational ages (GAs). These women were part of the Shengjing Birth Cohort (Birthcohorts 2017-05-10-0000-00-00). The serum samples were also stored at −80 °C.

Follow up and definition of the diagnoses in the prospective study

Follow up. All participants received systematic detailed ultrasound examinations at 22−26 weeks of gestation. Fetal echocardiography examinations were performed in all cases suspected to have fetal CHD. All neonates were examined by two pediatricians and follow-up assessments were performed at least six months after birth. Prenatal and neonatal findings were recorded.

Normal pregnancy with a healthy neonate. This cohort included deliveries beyond 37 weeks of gestation without any obstetric complications. All neonates were examined by two experienced neonatologists on the day of the delivery, and none of them were diagnosed with any congenital malformations prenatally and during the follow-up period.

Congenital structural abnormalities. Any major congenital structural abnormalities diagnosed antenatally by two antenatal sonographers with more than 10 years of experience, and/or after birth by surgery or autopsy. Abnormalities include CHD, NTD, cleft lip, urinary malformation, skeletal abnormalities, gastrointestinal tract malformations, and other defects. According to the European Surveillance of Congenital Anomalies guidelines, major congenital anomalies are defined as those which are lethal, carry high mortality risk or have other serious medical or functional consequences. The diseases included in the major malformations of each system are specified in a previous study.[12] Minor congenital malformations were excluded according to the exclusion guidelines of the European Surveillance of congenital anomalies.

Chromosomal abnormalities. Chromosomal abnormalities include those who were diagnosed antenatally via amniotic fluid karyotype analysis and/or high-throughput sequencing, and those who were diagnosed after birth via peripheral blood karyotype analysis and/or high-throughput sequencing.

Obstetric complications. Obstetric complications included PE, FGR, gestational hypertension, preterm delivery, gestational diabetes, and other complications (placental abruption, placenta previa, postpartum hemorrhage, and macrosomia). The diagnostic criteria for PE were the same as those in the case-control studies. FGR was defined as estimated fetal weight lower than the 10th percentile.[13] Gestational hypertension was defined as a systolic blood pressure of 140 mm Hg or more, or a diastolic blood pressure of 90 mm Hg or more, or both, on two occasions, at least four hours apart, after 20 weeks of gestation in a woman with a previously normal blood pressure.[14] Preterm delivery was defined as birth at less than 37 completed weeks of gestation.[15] Criteria for the diagnosis of gestational diabetes included, (1) Fasting plasma glucose ≥126 mg/dL (7.0 mmol/L); (2) 2-h plasma glucose ≥200 mg/dL (11.1 mmol/L) during oral glucose tolerance test; (3) Glycohemoglobin ≥6.5% (48 mmol/mol); (4) In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).[16] The diagnoses of other complications were also conducted in accordance with the ACOG.[17−19] In the case of two or more diagnoses, main indication for hospitalization was used as the categorizing criterion. For example, FGR due to gestational hypertension, we classified as gestational hypertension; macrosomia due to gestational diabetes, we classified as gestational diabetes.

Measurement of maternal serum LMNA via enzyme-linked immunosorbent assay (ELISA)

Maternal serum concentration of LMNA was measured using a commercially available sandwich ELISA kit (CUSABIO, Wuhan, China; Cat# EL013003HU) according to the manufacturers' instructions. The minimum detectable dose of human LMNA is typically less than 3.9 pg/mL. Quantifications were achieved by the construction of eight-point standard curves (0, 15·6, 31·2, 62·5, 125, 250, 500 and 1000 pg/mL) using known

concentrations of LMNA for each run. The R2 values of standard graphics were found to be greater than 0·99. The optical density of each well was measured with an Infinite M200 Pro/Nano Quant set to a wavelength of 450 nm (TECAN, Melbourne, Austria). The intra-assay precision and inter-assay precision were determined using the percent coefficient of variation. The coefficients of variation for intra- and inter-assay precisions were 3·7% and 4·5%, respectively. All assays were performed in triplicate, and the experimenters were carried out blindly to group assignment.

Ethics statement

All participants provided written informed consent to participate in the study, which was approved by the Ethics Committee of Shengjing Hospital (approval no. 2017PS264K).

Statistical analyses

Data were expressed as medians and inter quartile ranges (IQR) for continuous variables and as n (%) for the categorical data. In each case and control, the measured AFP, hCG, uE3, and LMNA values were converted into multiples of the median (MoM) after adjustment for GA. The Mann–Whitney U-test was performed to compare the differences among biomarkers in pregnancies with adverse outcomes and those with normal pregnancies. The Post-hoc Bonferroni correction was used for multiple comparisons. A Chi-square test or Fisher's exact test was used to compare the frequencies. Spearman correlation coefficients were used for correlation analysis. Receiver operating characteristic curves (ROCs) were analyzed to assess specificity and sensitivity of single candidate biomarkers and their combinations using binary logistic regression analysis. DeLong's test was used to compare AUCs from different models. The sample size of the case-control cohort was calculated using the sensitivity and specificity values obtained from the data in the prospective study. The number of patients in the CHD, NTD, and PE groups were 132, 152, and 323, respectively, thus providing a 95% statistical power at $P < 0·05$, 33, 38, and 81, respectively, 90% power at $P < 0·05$, and 15, 16, and 36, respectively, and an 85% power at $P < 0·05$. Statistical analyses were performed using the R statistics, Statistical Package for the Social Sciences (SPSS), version 22.0, MedCalc, version 11.4.2.0, and Graph Pad Prism, version 6.0. A P value of < 0.05 was considered statistically significant.

Role of funding source

Funding sources had no role in study design, data collection, data analyses, data interpretation, or writing of the report.

Results

Obstetric outcomes of the prospective study population

Between January 1, 2017 and June 30, 2018, a total of 2906 singleton pregnancies at 15–18 weeks of gestation were recruited. Of these, 195 were excluded due to hemolysis of their blood samples or a lack of consistent follow-up visits. The obstetric outcomes and clinical characteristics of the remaining 2,711 women are presented in Table 1. The congenital structural abnormalities, chromosomal abnormalities, and obstetric complications were observed in 152 (5·6%), 15 (0·6%), and 278 (10·3%) cases, respectively. Only in the PE high-risk assessment, there was a significant difference between the PE group and the normal group ($p < 0·0001$ by Chi-square test). The research design and workflow are depicted in Figure 1.

Correlation between LMNA expression and fetal GA, maternal age, BMI, and PE risk

Spearman correlation analysis was performed to determine the correlation between LMNA expression and fetal GA, maternal age, and maternal BMI in normal pregnancies. The serum LMNA level was significantly correlated with the fetal GA ($P < 0·0001$) but not with maternal age or BMI. There was no significant difference in the expression of LMNA between the PE high-risk women and PE low-risk women in both the control group ($p = 0·860$ by Mann-Whitney test) and PE group ($p = 0·401$ by Mann-Whitney test) (Figure 2). For each completed week of gestation from 15 to 18 weeks, the 50th percentile was taken as the median. The maternal serum LMNA values at each GA are presented in Table 2. In each case and control, the measured LMNA values were converted into MoM after adjustment for GA as previously described.

LMNA expression in adverse pregnancy outcomes

The median LMNA expression was significantly lower in CHD (median 0·53 MoM, IQR 0·33–0·68 MoM; $P < 0·0001$ by Mann-Whitney test) and NTD (median 0·41 MoM, IQR 0·34–0·66 MoM; $P < 0·0001$ by Mann-Whitney test) groups, but not in the other group of structural abnormalities. There was no significant difference in LMNA expression between the group of chromosomal abnormalities and the healthy group. Among the groups with obstetric complications, only the PE group demonstrated a significant downregulation in the LMNA level (median 0·65 MoM, IQR 0·44–0·98 MoM; $P < 0·0001$ by Mann-Whitney test) compared with the control group (Figure 3).

Comparison of LMNA with traditional biomarkers

The results of the ROC curve analysis demonstrated an area under the curve (AUC) of 0·933 (95% CI,

Obstetric outcomes	n (%)	Maternal age (years)	*p*	BMI	*p*	PE high risk	*p*
Uncomplicated delivery of a term healthy neonate	2276 (83·65%)	29·00 (27·00—30·00)		22·45 (20·20—24·98)		26 (1·14%)	
Congenital structural abnormalities	152 (5·59%)						
Congenital heart defects	21 (0·77 %)	28·00 (26·00—29·00)	0·103	23·59 (19·27—26·86)	0·573	0	1·000
Neural tube defects	9 (0·33 %)	30·00 (29·00—32·50)	0·062	24·61 (21·02—25·64)	0·453	0	1·000
Lip cleft	16 (0·59%)	28·50 (26·00—31·00)	0·943	23·57 (21·33—26·08)	0·271	1 (6·25%)	0·173
Urinary malformations	20 (0·70%)	29·00 (26·00—32·00)	0·618	21·50 (20·64—26·52)	0·861	0	1·000
Skeletal abnormalities	11 (0·40%)	29·00 (26·00—32·00)	0·053	21·60 (19·13—24·77)	0·510	0	1·000
Gastrointestinal tract malformations	15 (0·55%)	28·00 (25·00—33·00)	0·956	23·42 (20·76—26·08)	0·538	1 (6·67%)	0·163
Others	60 (2·21%)	29·00 (26·25—30·75)	0·980	22·91 (20·48—26·90)	0·074	2 (3·33%)	0·161
Chromosomal abnormalities	15 (0·55%)	27·00 (26·00—30·00)	0·276	24·22 (19·84—27·73)	0·172	0	1·000
Obstetric complications	278 (10.22%)						
Preeclampsia	30 (1·10%)	28·00 (26·00—29·25)	0·158	22·72 (20·60—24·96)	0·679	11 (36·7%)	<0·0001
Fetal growth restriction	46 (1·69%)	28·50 (27·00—31·00)	0·970	22·04 (19·92—24·85)	0·487	1 (2·17%)	1·000
Gestational hypertension	65 (2·39%)	28·00 (25·50—30·00)	0·057	22·14 (20·32—24·53)	0·507	2 (3·08%)	0·181
Preterm delivery	49 (1·80%)	28·00 (26·00—31·00)	0·487	22·58 (20·14—25·67)	0·790	1 (2·04%)	1·000
Gestational diabetes	21 (0·77%)	29·00 (25·00—31·00)	0·666	22·86 (20·60—25·53)	0·575	0	1·000
Others	67 (2·46%)	27·00 (26·00—30·00)	0·069	22·50 (19·84—24·97)	0·419	2 (2·99%)	0·190
Total	2721 (100%)						

***Table 1*: Obstetric outcomes and clinical characteristics of the prospective study population.**
Data are n (%), or median (interquartile ranges). P values were determined by Mann-Whitney test or Chi-square test compared with uncomplicated delivery group. *Abbreviations*: BMI, body mass index.

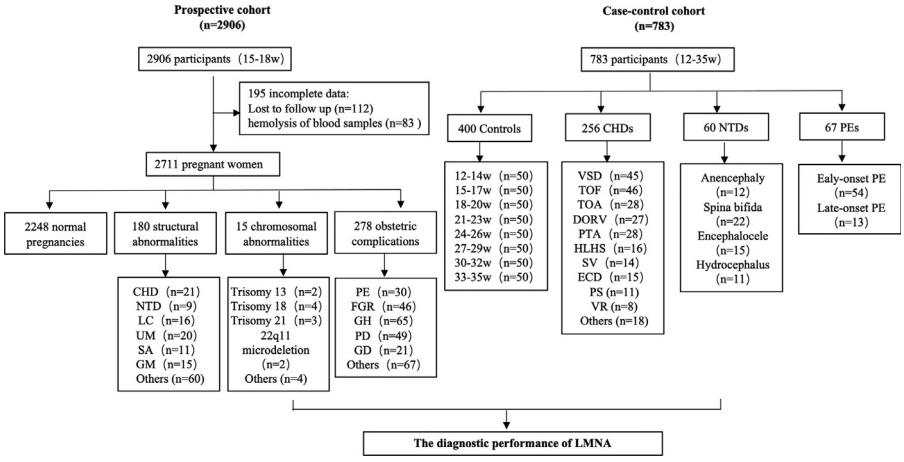

Figure 1. Overall workflow and cohort information of the study.

Abbreviations: CHD, congenital heart defects; DORV, double outlet right ventricle; ECD, endocardial cushion defect; FGR, fetal growth restriction; GD, gestational diabetes; GH, gestational hypertension; GM, gastrointestinal tract malformations; HLHS, Hypoplastic left heart syndrome; LC, lip cleft; NTD, neural tube defects; PD, preterm delivery; PE, preeclampsia; PS, pulmonary stenosis; PTA, persistent truncus arteriosus; SA, skeletal abnormalities; SV, single ventricle; TOA, transposition of great arteries; TOF, tetralogy of Fallot; UM, urinary malformations; VR, vascular ring VSD, ventricular septal defect.

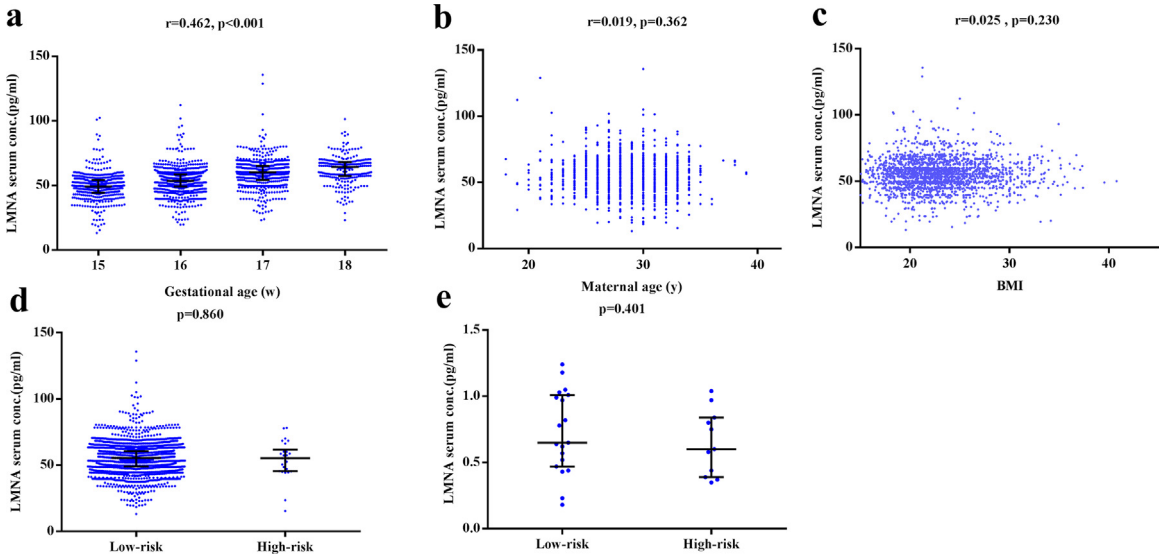

Figure 2. The correlation between LMNA expression and fetal gestational age, maternal age, BMI, and PE risk. (a–c) ELISA analysis was performed on the normal pregnancies (uncomplicated delivery of a term healthy neonate) in the prospective study (n = 2276). P values were determined by Spearman's correlation analysis. (d) In the normal pregnancies of the prospective study, the expression of LMNA in the PE high-risk group (n = 26) and PE low-risk group (n = 2250) was compared by Mann-Whitney test. (e) ELISA analysis was performed on the PE pregnancies in the prospective study (n = 30), and the expression of LMNA in the PE high-risk group (n = 11) and PE low-risk group (n = 19) was compared by Mann-Whitney test. Data is expressed as rank correlation coefficient (r) or median and interquartile ranges. ELISA measurements were performed in triplicate.

Abbreviations: BMI, body mass index; PE, preeclampsia.

0·922–0·943) for LMNA to detect CHD. The overall test sensitivity was 90·5% (95% CI, 69·6–98·8%), while the specificity was 89·3% (95% CI, 88·0–90·6%). The AUC for AFP, hCG, and uE3 were 0·527, 0·525, and 0·530, respectively, in detecting CHD (Figure 4a). The AUC for LMNA was significantly greater than the AUC for AFP, hCG, and uE3 (all P < 0·0001 by DeLong's test). Combinations of the

Gestational age(w)	Median (pg/ml)	10th percentile (pg/ml)	90th percentile (pg/ml)	10th percentile /median	*n*
15	49·2	38·4	58·7	0·78	501
16	53·7	43·0	63·2	0·80	900
17	60·2	47·5	70·1	0·79	590
18	64·7	48·5	73·9	0·75	285

***Table 2*: LMNA expression in normal pregnancy (15−18 w).**

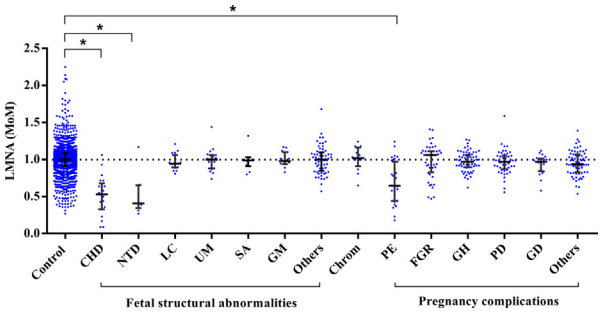

Figure 3. LMNA expression in adverse pregnancy outcomes in the prospective study. ELISA analysis was performed on the women with adverse pregnancy outcomes, and in women with normal pregnancies (n = 2276 for normal pregnancies; n = 21 for CHD; n = 9 for NTD; n = 16 for LC; n = 20 for UM; n = 11 for SA; n = 15 for GM; n = 60 for other congenital structural abnormalities; n = 15 for Chrom; n = 30 for PE; n = 46 for FGR; n = 65 for GH; n = 49 for PD; n = 21 for GD; n = 67 for other obstetric complications.) * represents $P < 0.0001$ by Mann-Whitney test. Data is expressed as median and interquartile ranges. ELISA measurements were performed in triplicate.

Abbreviations: CHD, congenital heart defects; Chrom, chromosomal abnormalities; FGR, fetal growth restriction; GD, gestational diabetes; GH, gestational hypertension; GM, gastrointestinal tract malformations; LC, lip cleft; NTD, neural tube defects; PD, preterm delivery; PE, preeclampsia; SA, skeletal abnormalities; UM, urinary malformations.

proteins did not improve the diagnostic performance (p = 0·525 by DeLong's test) compared with LMNA alone.

The ROC curve analysis of LMNA demonstrated an increased sensitivity and specificity to detect NTD with an AUC of 0·890 (95% CI, 0·876−0·902) compared with that of AFP (0·829) (95% CI, 0·813−0·844, p = 0·672 by DeLong's test). The combination of both proteins outperformed AFP with an AUC of 0·990 (95% CI, 0·985−0·993, p = 0·047 by DeLong's test) (Figure 4b). NTD could be predicted with a sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio of 100%, 95·0%, 20·1, and 0·0, respectively.

For the prediction of PE, the AUC was 0·790 (95% CI, 0·772−0·806), with a sensitivity of 70·0% and a specificity of 82·4%. In comparison, the performance of AFP, hCG, and uE3 for predicting PE was poor, with AUCs of 0·559, 0·568, and 0·604, respectively (Figure 4c). The AUC for LMNA was significantly greater than the AUC for AFP (p = 0·003), hCG (p = 0·008), or uE3 (p = 0·023 by DeLong's test). Their combinations did not improve the diagnostic performance (p = 0·686 by DeLong's test) compared with LMNA alone. The performance of LMNA was better in predicting early-onset PE, with an AUC of 0·941 (95% CI, 0·930−0·950) and a sensitivity and specificity of 94·4% and 88·5%, respectively (Figure 4d). Among the 30 pregnant women with PE, 26 cases were screened at 15−16 weeks, and the AUC value of LMNA for PE diagnosis before 16 weeks was 0·818 (95% CI, 0·797−0·838) (Figure 4e).

We further performed ROC curve analysis to evaluate the diagnostic value of LMNA for CHD, NTD, and PE in the entire cohort. The AUC for LMNA to detect CHD, NTD, and PE in the entire cohort was 0·853 (95% CI, 0·839−0·866). The overall test sensitivity was 76·7% (95% CI, 64·0−86·6%), while the specificity was 88·4% (95% CI, 87·1−89·5%) (Figure 4f).

Case-control study population and LMNA expression at different GAs

Serum samples from 256 women with CHD fetuses, 60 with NTD fetuses, and 67 women with PE were collected for the case-control study. This sample size provided 95% statistical power at $p < 0·05$ to differentiate CHD, 90% power at $p < 0·05$ to differentiate NTD, and 85% power at $p < 0·05$ to differentiate PE from normal pregnancies. As the gestational age in the case group ranged from 12 to 35 weeks, 400 women carrying healthy fetuses at the corresponding GAs (12 to 35 weeks) from Shengjing Birth Cohort were included as

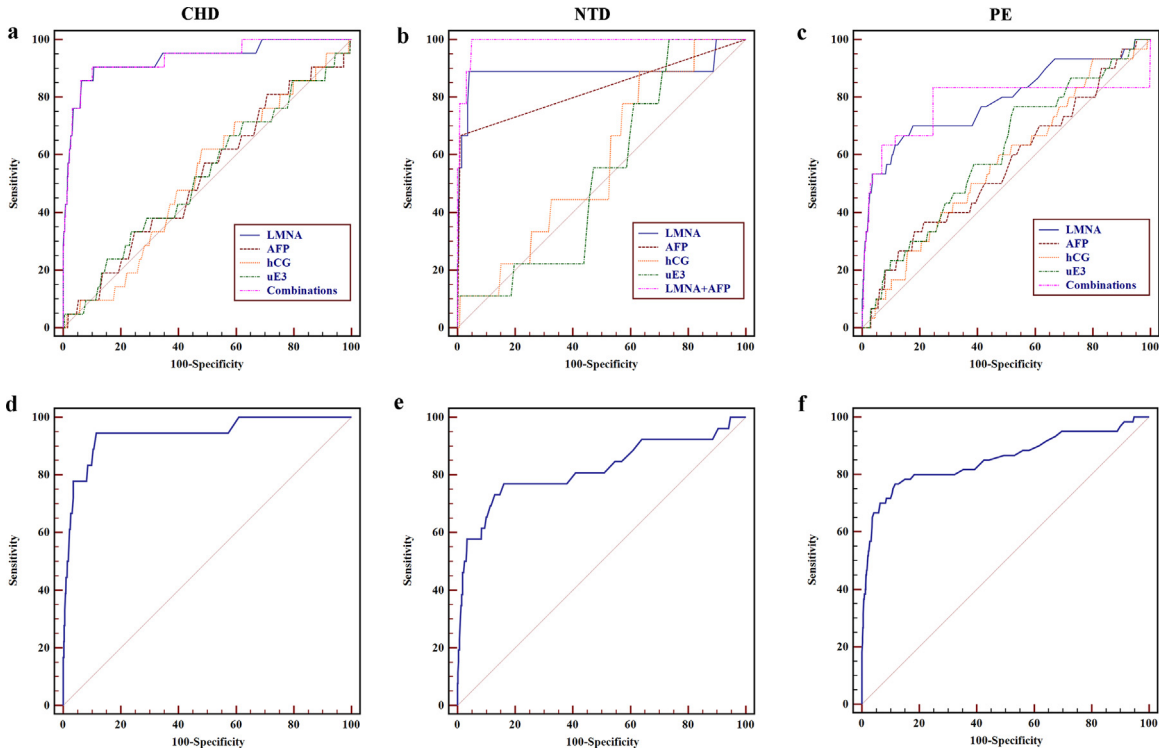

Figure 4. Comparison of LMNA with traditional biomarkers. (a-c) The ROC curves of LMNA and its combination with traditional biomarkers (AFP, hCG, and uE3) for distinguishing pregnant women carrying fetuses with CHD, NTD, and PE from healthy fetuses (Normal group, *n* = 2276; CHD group, *n* = 21; NTD group, *n* = 9; PE group, *n* = 30). (d) The ROC curve of LMNA in prenatal diagnosis of early-onset PE (Normal group, *n* = 2276; early-onset PE group, *n* = 18). (e) The ROC curve of LMNA in prenatal diagnosis of PE at 15−16 weeks (Normal group, *n* = 1401; PE group, *n* = 26). (f) The ROC curve of LMNA in prenatal diagnosis of CHD, NTD and PE in the entire cohort (CHD, NTD, and PE group, *n* = 60; other pregnancy outcome group, *n* = 2661).

Abbreviations: CHD, congenital heart defects; NTD, neural tube defects; PE, preeclampsia; ROC, receiver operating characteristic.

the control group. Because of the wide range of GAs, we converted the measured LMNA concentrations into MoMs to offset the variation in expression caused by different GAs. In order to reduce the effect of individual differences on the median value calculation, results over three-week gestational periods were combined (12−14, 15−17,18−20, 21−23, 24−26, 27−29, 30−32, and 33−35 weeks), and we assigned 50 controls for each three-week period (Figure 1). The 50th percentile in each period was taken as the median. In each case and corresponding control, the measured LMNA values were converted into MoM as described previously. The clinical characteristics of the study population in the case-control study are presented in Table 3. During normal pregnancy, the LMNA content started to increase from 40·35 pg/mL at 12 weeks, and to 69.20 pg/mL at 24 weeks, before falling gradually to 46·30 pg/mL at 35 weeks of gestation (Supplemental Figure 1).

Diagnostic performance of LMNA in the case-control study

There were significant differences between adverse pregnancies (CHD, NTD, and PE) and normal pregnancies in the vast majority of GA ranges (Figure 5). Even in the 12−14 weeks group, maternal serum LMNA

	n	Maternal age (years)	*p*	BMI	*p*	PE high risk	*p*
Control	400	28·00(26·00−31·00)		22·83(21·01−25·34)		0	
CHD	256	29·0(27·00−21·00)	0·100	22·71(20·78−25·36)	0·564	0	1·000
NTD	60	28·00(26·00−30·00)	0·995	23·14(20·47−25·11)	0·966	0	1·000
PE	67	29·00(26·00−31·00)	0·507	22·77(20·42−25·39)	0·801	21(31·34%)	<0·0001

***Table 3*: Clinical characteristics of the study population in the case-control study.**
Data are n (%), or median (IQR). *Abbreviations*: BMI, body mass index.

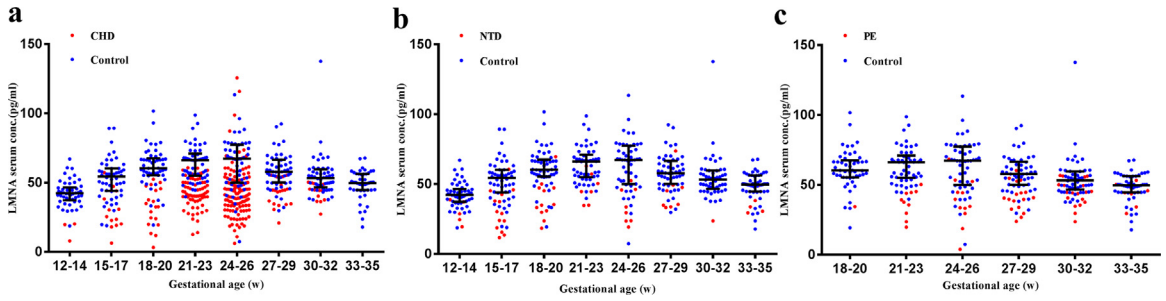

Figure 5. Differences between adverse pregnancies (CHD, NTD, and PE) and normal pregnancies at different gestational ages in the case-control study. ELISA analysis was performed on women with CHD (n = 256), NTD (n = 60), PE (n = 67), and normal pregnancies (n = 400). The expression of LMNA in women with adverse pregnancy outcomes and in women with normal pregnancies was compared by Mann−Whitney test. Data in control group is expressed as median and interquartile ranges, and data in CHD, NTD, and PE group is expressed as scatter dot plot. ELISA measurements were performed in triplicate.

Abbreviations: CHD, congenital heart defects; NTD, neural tube defects; PE, preeclampsia.

showed significant decreased expression in both CHD and NTD groups (p < 0·0001 by Mann-Whitney test) compared to the control.

LMNA was significantly downregulated in all the sub-groups of CHDs, except in the pulmonary stenosis (PS) (p = 0·301 by Post-hoc Bonferroni correction) and vascular ring (VR) (p = 1·000 by Post-hoc Bonferroni correction) groups (Figure 6a). The ROC curve revealed an AUC of 0·875 for LMNA in prenatal diagnosis of CHD, with a sensitivity of 85·5% and a specificity of 76·2%, and at a cut-off value of 0·85 MoM (Table 4).

Among the sub-groups of NTDs, the median LMNA MoM was significantly lower than that of the control group (Figure 6b). The AUC was 0·871, with a sensitivity and specificity of 86·7% and 76·2%, respectively, and at a cut-off value of 0·85 MoM (Table 4).

LMNA expression was also downregulated in PE pregnancies, and there was further statistical difference in LMNA values between early- and late-onset PE (p = 0·013 by Post-hoc Bonferroni correction) (Figure 6c). The performance of LMNA was better in predicting early-onset PE (AUC, 0·851) than at predicting late-onset PE (AUC, 0·674) (Table 4).

Discussion

LMNA is a nuclear intermediate filament protein critical for nuclear architecture and mechanics.[20] Mutations in *LMNA* are linked to a spectrum of genetic diseases ranging from cardiomyopathy to lipodystrophy and progeria, which are termed as laminopathies.[21] Our previous study revealed that LMNA could serve as a potential

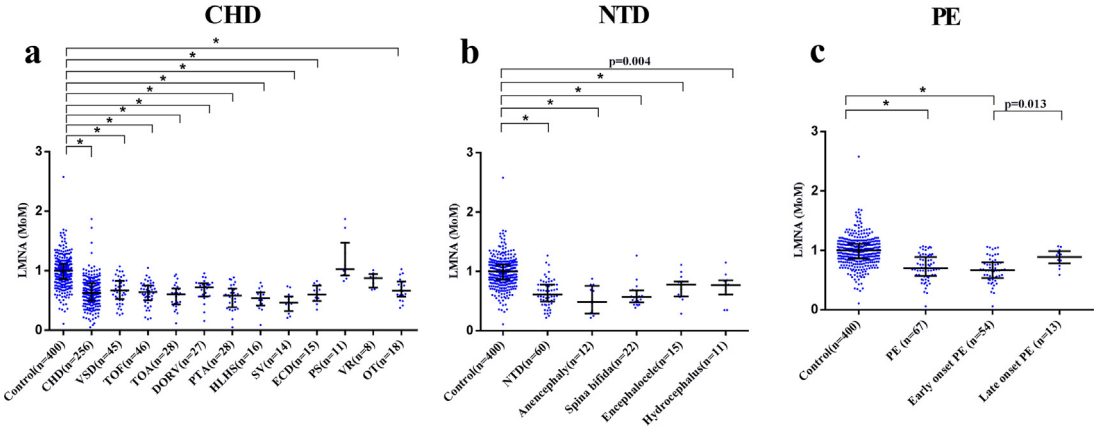

Figure 6. LMNA expression in different subtypes of CHD, NTD, and PE in the case-control study. The expression of LMNA among each subtype of CHD, NTD, and PE groups and the control group were compared by Post-hoc Bonferroni correction. Data is expressed as median and interquartile ranges. * represents P < 0.0001.

Abbreviations: CHD, congenital heart defects; DORV, double outlet right ventricle; ECD, endocardial cushion defect; HLHS, hypoplastic left heart syndrome; NTD, neural tube defects; OT, other congenital heart defects; PE, preeclampsia; PS, pulmonary stenosis; PTA, persistent truncus arteriosus; SV, single ventricle; TOA, transposition of great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect; VR, vascular ring.

	AUC	Sensitivity	Specificity	LRP	LRN	Cut-off
CHD	0·875	85·55	76·25	3·60	0·19	0·85
NTD	0·871	86·67	76·25	3·65	0·17	0·85
Early onset PE	0·851	75·93	83·50	4·60	0·29	0·79
Late onset PE	0·674	76·90	58·00	1·83	0·40	0·96

***Table 4*: Diagnostic performance of LMNA in prenatal prediction of CHD, NTD, and PE in the case-control study.**
Abbreviations: AUC, area under curve; CHD, congenital heart defects; LRN, negative likelihood ratio; LRP, positive likelihood ratio; NTD, neural tube defects; PE, preeclampsia.

independent biomarker in prenatal diagnosis of CHD.[9] In this study, we found that the expression of LMNA in maternal serum was correlated with GAs in normal pregnancies, which indicated that LMNA was a pregnancy associated marker whose differential expression might reflect some changes in fetal development and maternal health. We further discovered that the downregulation of LMNA in maternal serum could not only predict CHD but also NTD and PE antenatally.

Currently, there are no known biomarkers for antenatal CHD screening despite it being one of the most common congenital birth defects. Clinical strategies to diagnose CHD mostly depend on fetal echocardiography, which is often resource-limited and is only offered to high-risk mothers. According to a multicenter study done in China, despite the high specificity of fetal echocardiography at 99·8%, the sensitivity was far lower, at only 33·9%.[22] Therefore, serum biomarkers for CHD screening are urgently needed. Alanen et al. studied the diagnostic performance of traditional biomarkers (PAPP-A and β-hCG) in prenatal diagnosis of CHD and found that both PAPP-A and β-hCG levels were lower in severe cases of CHD.[23] In this study, we also evaluated the expression of β-hCG in the CHD group; however, we did not find a significant difference. The difference in the results of the two studies may be explained by the different subtypes of CHD. The former included only severe CHDs, while our study included almost all subtypes of CHDs. In our previous study, we performed a comprehensive maternal serum proteomics assessment combined with immunoassays to identify non-invasive biomarkers for the prenatal diagnosis of CHDs. LMNA was the best diagnostic biomarker at 22 to 26 weeks of gestation.[9] In this study, we used two independent cohorts and a larger sample size to verify the diagnostic value of LMNA for CHD. We found that LMNA was significantly downregulated in all the subgroups of CHDs, except in the pulmonary stenosis and vascular ring groups. Moreover, the GA range of the fetuses included in this study was between 12 and 35 weeks; and our results suggested that CHD could be predicted by maternal serum LMNA level as early as the first trimester. If the LMNA test could be added to the serum screening of the first or second trimester, serological screening of CHD could be enhanced, allowing for earlier detection of CHD.

AFP has been a traditional diagnostic biomarker in the prenatal diagnosis of NTD, but its false positive rate is high. According to one study from China, the positive predictive value of AFP for NTD diagnosis was only 16·2%.[24] In recent years, several studies have been conducted to identify new NTD markers, but none has been translated into clinical applications.[25,26] In this study, the diagnostic accuracy of LMNA for NTD prediction was higher than that of AFP, and their combination did significantly improve the diagnostic efficiency (AUC, 0·990). Besides, the diagnosis of NTD by LMNA was not affected by the subtypes. Application of the LMNA test to serum screening performed in the first or second trimesters, and combination with AFP, could ultimately improve the diagnostic accuracy of NTD.

We found that LMNA is also a predictor of PE, which is a leading cause of maternal and neonatal mortality. Although several screening models combining serum biomarkers, clinical characteristics, and ultrasound parameters have been developed to identify pregnancies at high risk for PE, they have performed poorly when applied to populations other than the population from which they were derived.[27,28] The expression of LMNA in PE patients is independent of the risk assessment results in the first trimester. Therefore, our newly discovered biomarker, LMNA, is expected to improve the diagnostic accuracy of PE in combination with traditional indicators, though future studies should validate these results with larger sample sizes. Furthermore, aspirin initiated before 16 weeks of gestation has been associated with a significant reduction of preterm PE.[29] Our results showed that detection of LMNA concentration in maternal serum before 16 weeks of gestation was expected to predict PE, which has important clinical significance for early diagnosis and treatment of PE.

How to explain the down-regulation of LMNA in these adverse pregnancies? LMNA is the main constituent of the nuclear cytoskeleton, acting as a scaffold for protein complexes that regulate nuclear structure and functions and which potentially participates in signal transduction by mediating movements between the cytoplasm and the nucleus.[30–32] Constantinescu et al. studied LMNA expression in human embryonic stem cells and found that it was expressed in these cells upon differentiation into neuronal lineages and cardiomyocytes.[33] In addition, both CHD and NTD were related to

the abnormal neural crest development during embryogenesis.[34,35] This could explain the decreased expression of LMNA in NTD and CHD pregnancies. Furthermore, Xin et al. found that LMNA was downregulated in the placental villi of women who had early pregnancy loss, which also indicated that LMNA played an important role in placental function.[36] In our study, the expression of LMNA in early-onset PE was lower than that in late-onset PE. Early-onset PE is mainly related to impaired placentation with insufficient trophoblast invasion, leading to impaired uterine spiral artery remodeling and angiogenesis, whereas late-onset PE is more closely related to maternal microvascular disease,[37] which could explain why the expression of LMNA was more significantly downregulated in early-onset PE. However, the biological role of LMNA in pregnancy and embryo development needs further exploration.

In this study, the AUC in the case-control cohort was slightly lower than that in the prospective cohort, which could be explained by one of two reasons. The first is due to the individual differences between two independent samples. Second, we found that the serum LMNA level of healthy pregnant women increased starting around 12 weeks, and gradually decreased after 24 weeks. This suggests that the protein may play a more significant role in embryonic development before 24 weeks, resulting in a more pronounced difference in early pregnancy.

The advantage of this study is the detection of LMNA in a prospective cohort that included multiple types and subtypes of adverse pregnancies. The prospective cohort in this study was derived from random selection of pregnant women screened for Down's syndrome in the second trimester, and the incidence of various adverse pregnancies were close to those reported in the literature. We found several adverse pregnancy outcomes related to the differential expression of LMNA, and we validated these findings in an independent case-control cohort that met the requirements of diagnostic experiments with respect to the sample size for abnormal cases, and clarified the diagnostic value of LMNA in several adverse pregnancy outcomes. In addition, we included cases that covered different GAs, evaluated the applicable GAs for LMNA testing, and used the calculated MoM value to correct for the differences in GA. However, this study also had several limitations. First, this is a single-center study, therefore a multi-center study with a larger sample size is expected in the future. In addition, we look forward to the development of a multi-parameter algorithm that combines LMNA, traditional biomarkers, and patient clinical information to more accurately predict different adverse pregnancy outcomes.

Given the possible predictive value of LMNA for adverse pregnancy outcomes, it could be a promising potential biomarker for the prenatal diagnosis of CHD, NTD, and PE. Based on the predictive value of LMNA for these adverse pregnancy outcomes at different gestational ages, we expected that it could be added to serological screening in the first trimester; however, the predictive value of LMNA in early pregnancy for these adverse pregnancy outcomes needs to be further confirmed in studies featuring larger sample sizes. Following detection of decreased LMNA expression, prenatal ultrasound examination and fetal cardiogram examination should be offered to rule out CHD and NTD. If the ultrasound examinations appear normal with respect to CHD and NTD, clinicians should still be aware of the possibility of the occurrence of PE. Combined with other predictors of PE, preventive measures such as a regimen of low-dose aspirin should be considered. Such an approach would be of great significance for the earlier and more accurate screening of CHD, NTD, and PE in the clinical setting.

Declaration of interests

All authors report no conflict of interest.

Contributions

LZC, QJW, and ZWY designed the experiments. YX, YW, JX, and QBW collected blood sample, and prepared plasma. LZC, YW, and YXZ performed experiments for the study. LZC, YX, QJW, and YXZ collected and analyzed the data. LZC and ZWY wrote the first draft of the paper. LZC, ZWY, YX, and QJW contributed to the writing of the paper. All authors read and approved the final manuscript.

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Data sharing statement

The related data and materials are available for sharing upon request to Prof. Lizhu Chen and Prof. Zhengwei Yuan.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.103932.

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