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Abstract—Preeclampsia is a major determinant of fetal and maternal morbidity and mortality. We used a proteomic strategy to identify urinary biomarkers that predict preeclampsia before the onset of disease. We prospectively collected urine samples from women throughout pregnancy. Samples from gestational weeks 12 to 16 (n=45), 20 (n=50), and 28 (n=18) from women who subsequently had preeclampsia develop were matched to controls (n=86, n=49, and n=17, respectively). We performed capillary electrophoresis online coupled to micro-time-of-flight mass spectrometry. Disease-specific peptide patterns were generated using support vector machine-based software. Candidate biomarkers were sequenced by liquid chromatography-tandem mass spectrometry. From comparison with nonpregnant controls, we defined a panel of 284 pregnancy-specific proteomic biomarkers. Subsequently, we developed a model of 50 biomarkers from specimens obtained at week 28 that was associated with future preeclampsia (classification factor in cases, 1.032±0.411 vs controls, −1.038±0.432; $P<0.001$). Classification factor increased markedly from week 12 to 16 to 28 in women who subsequently had preeclampsia develop (n=16; from −0.392±0.383 to 1.070±0.383; $P<0.001$) and decreased slightly in controls (n=16; from −0.647±0.437 to −1.024±0.433; $P=0.043$). Among the biomarkers are fibrinogen alpha chain, collagen alpha chain, and uromodulin fragments. The markers appear to predict preeclampsia at gestational week 28 with good confidence but not reliably at earlier time points (weeks 12–16 and 20). After prospective validation in other cohorts, these markers may contribute to better prediction, monitoring, and accurate diagnosis of preeclampsia. **(*Hypertension*. 2011;57[part 2]:561-569.) ● Online Data Supplement**

Key Words: biomarkers ■ extracellular matrix ■ preeclampsia ■ pregnancy ■ proteomics

Preeclampsia affects 3% to 7% of pregnant women worldwide and represents a significant challenge for the scientific community. Several maternal risk factors are associated with the condition,[1] but it remains extremely difficult to predict which women are likely to be affected. Once diagnosed, there is no effective treatment other than delivery of the baby. Accurate identification of those at risk would facilitate intervention such as low-dose aspirin and/or calcium supplementation,[2,3] closer monitoring, and timely intervention when required. In addition to clinical characteristics, several biochemical markers including serum FMS-like tyrosine kinase-1 and placental growth factor have been proposed for improving early and accurate prediction and diagnosis of preeclampsia;[4] however, none has been sensitive or specific enough to be used routinely in clinical practice.

The majority of biomarkers investigated for the prediction of preeclampsia have been hypothesis-led and derived from pathways implicated in the pathogenesis of the condition. It is probably unrealistic to expect that a single biomarker could be used to predict such a diverse condition as preeclampsia. Research in recent years therefore has moved toward unbiased "systems medicine" approaches, using hypothesis-generating strategies to investigate new pathways. One such approach that holds promise in preeclampsia research is proteomics, the analysis of thousands of peptides and proteins simultaneously.[5]

Urine represents a promising medium for proteomic-based preeclampsia research. Renal pathology is one of the hallmarks of the condition and, in keeping with serum, urinary levels of antiangiogenic peptides have been shown to be altered in women with preeclampsia.[6,7] Urine is stable when frozen at −20°C or −70°C for several years, without requiring special preparation.[8] We and others have identified and validated urinary proteomic biomarkers for the early diagnosis of various diseases, including coronary artery disease,[9,10] diabetes,[11] and diabetic nephropathy.[12] In addition to providing potentially clinically useful biomarkers, these studies also have given insight to novel pathophysiological mechanisms. Encouraged by these observations, we used capillary-electrophoresis (CE) mass spectrometry (MS) techniques to

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Table 1. Characteristics of the Proteomics in Preeclampsia Study Cohort

Characteristic	Preeclampsia, n=45	No Preeclampsia, n=2340	*P*
Maternal characteristics			
Age (y)	29±5	30±6	0.06
Ethnicity			
White	42 (93%)	2133 (91%)	0.59
Other	3 (7%)	207 (9%)	
Body mass index (kg/m^2)	29±5	26±5	<0.001
Nulliparous	39 (87%)	1176 (50%)	<0.001
Smoker	4 (9%)	282 (12%)	0.65
Previous preeclampsia	1 (2%)	75 (3%)	1.00
Family history of preeclampsia (mother or sister) at booking	8 (17%)	109 (5%)	0.001
Systolic blood pressure (mm Hg)	121±10	111±13	0.01
Diastolic blood pressure (mm Hg)	76±9	67±10	0.002
Proteinuria (≥+ on dipstick)	1 (2%)	34 (1%)	0.48
Gestation at sampling (wk)	13.8±1.6	13.7±1.7	0.94
Pregnancy outcome			
Cesarean section	20 (44%)	325 (14%)	<0.001
Gestation at delivery (wk)	38.3±2.0	39.7±1.8	<0.001
Birth weight (g)	3155±621	3443±565	<0.001

All data are mean±SD or N (% of total) from the study visit at gestational week 16–18. Comparisons between cases and controls were made using Student *t* test or Fisher exact test as appropriate.

examine the urinary proteome in pregnancy and to investigate peptide patterns that could be used as predictive biomarkers for preeclampsia.

Patients and Methods

Patient Recruitment

Samples from 2 separate study populations were examined. The Proteomics in Preeclampsia (PIP) study was a longitudinal study designed to identify urinary proteomic biomarkers in early pregnancy that could be used to predict preeclampsia in later pregnancy. Two-thousand five hundred women with singleton pregnancies were recruited by study nurses at their initial antenatal hospital visit (gestational week 12–16) at the Southern General Hospital, the Queen Mother's Hospital, and the Princess Royal Maternity Hospital, Glasgow, between October 2007 and March 2009. Women with a history of chronic hypertension, diabetes, or renal disease were excluded. After written informed consent, urine and blood samples, and information about medical and obstetric history were obtained. Delivery information, obtained from hospital databases, was available for 2407 women (95%); those who had fetal losses at <20 weeks of gestation (n=22) were not included, leaving 2385 women in the final analysis. Case notes were reviewed by 1 investigator (D.M.C.) for all women who had gestational hypertension or preeclampsia develop. Characteristics of the overall study population are shown in Table 1.

To identify biomarkers in mid pregnancy that could be used to predict preeclampsia, 132 of the 2385 women who were identified as having at least 2 traditional risk factors for preeclampsia[1] attended for further sampling at gestational week 28. Of the 132 women, 18 (13.6%) had preeclampsia develop and were matched for age and body mass index at sampling to 17 controls who had normotensive deliveries of appropriately grown babies after 37 weeks of gestation. Patient characteristics are shown in Supplemental Table S1 (please see http://hyper.ahajournals.org).

Overall, 45 (1.9%) of the 2385 women in the PIP study had preeclampsia develop (cases). Cases were matched for age, body mass index, and gestational age at sampling with 86 women who had uncomplicated pregnancies (controls) using a nested case-control design. Patient characteristics are shown in Supplemental Table S2 (please see http://hyper.ahajournals.org). Of the 45 cases, 12 had proteinuria confirmed either by 24-hour collection or by protein-to-creatinine ratio, and the remainder had dipstick-positive proteinuria. Eighty percent of the 45 women with preeclampsia delivered after 37 weeks of gestation; none had documented hypertension before 28 weeks of gestation.

Further early pregnancy urinary samples were obtained from the Screening for Pregnancy Endpoints (SCOPE) study, an international cohort study of healthy nulliparous women with singleton pregnancies.[13] Between November 2004 and October 2008, 3234 women were recruited into the SCOPE study in Auckland, New Zealand, and in Adelaide, Australia. Women were interviewed, examined, and specimens obtained at 15±1 and 20±1 weeks of gestation and had an ultrasound scan at 20±1 weeks of gestation. Women were followed-up prospectively throughout pregnancy, with outcome data collected by research midwives. All preeclampsia cases were reviewed by the principal investigators and the diagnosis was confirmed. Data were entered directly into a web-based database with a complete audit trail (MedSciNet). Pregnancy outcome data were available for 99% (n=3196). After exclusion of fetal losses before 22 weeks of gestation (n=26) and women not attending the 20-week interview (n=69), the base population for this study comprised 3101 women (96% of recruits). Of the 3101 women, 175 (5.6%) had preeclampsia develop, of whom 158 (5.1%) delivered after 34 weeks of gestation. SCOPE cases (n=50) were randomly selected from the late-onset preeclampsia subgroup and controls (n=50) were selected from women with uncomplicated pregnancies (n=1767), defined as no antenatal obstetric or medical complications with delivery of an appropriately grown healthy baby at ≥37 weeks of gestation. Among the 50 women with preeclampsia, 45 had proteinuria and 5 had preeclampsia diagnosed after multi-organ complications developed. Urinary samples from gestational week 20 were used for proteomic analysis, with 1 control specimen excluded for technical reasons. Patient characteristics are shown in Supplementary Table S3 (please see http://hyper.ahajournals.org).

Preeclampsia was defined as blood pressure ≥140/90 mm Hg after 20 weeks of gestation (but before the onset of labor) or in the postnatal period, with either proteinuria (24-hour urinary protein ≥300 mg, spot urine protein-to-creatinine ratio ≥30 mg/mmol, or urine dipstick ≥++) or evidence of multi-organ complications or both.[14]

To characterize the urinary proteome in normal pregnancy, the results from pregnant women were compared with the urinary proteome in apparently healthy, nonpregnant, female employees of the University of Hannover, Germany, recruited in 2002 to 2003. Samples from 17 women with uncomplicated pregnancies (PIP study, gestational week 28) were compared with samples from 67 nonpregnant women aged 28±5 years for biomarker discovery. A further 248 samples from healthy pregnant women and 141 samples from apparently healthy nonpregnant women aged 34±12 years were used for validation of the pregnancy-specific urinary proteomic panel.

All women provided written informed consent; PIP research protocols were approved by the West Glasgow Research Ethics Committee (07/S0709/79) and SCOPE protocols were approved by New Zealand (AKX/02/00/364) and Australia (1712/5/2008) Research Ethics Committees. Both studies adhere to the principles of the Declaration of Helsinki.

Sample Handling

Midstream urine samples were collected at the outpatient clinic (PIP) or study interview (SCOPE), separated, and stored in aliquots within

4 hours. Samples were frozen at −70°C and were thawed immediately before analysis.

For each sample, a 0.7-mL aliquot of urine was diluted with 0.7 mL of 2 mol/L urea and 10 mmol/L NH_4OH containing 0.02% SDS. To remove proteins with a molecular mass >20 kDa such as albumin, samples were filtered using Centrisart ultracentrifugation filter devices (Sartorius, Göttingen, Germany) at 3000*g* until 1.1 mL of filtrate was obtained. To remove urea, electrolytes, and salts, and to enrich the polypeptides present, the filtrate was then applied onto a PD-10 desalting column (Amersham Bioscience) equilibrated in 0.01% NH_4OH in high-performance liquid chromatography-grade H_2O (Carl Roth). Finally, all samples were lyophilized, stored at 4°C, and resuspended in high-performance liquid chromatography-grade H_2O before analysis.

CE-MS analysis was performed as previously described[15,16] using a P/ACE MDQ capillary electrophoresis system (Beckman Coulter) online coupled to a time-of-flight mass spectrometer (micro-time-of-flight MS; Bruker Daltonic). Data acquisition and MS acquisition methods were automatically controlled by the CE via contact close relays. Spectra were accumulated every 3 seconds over a range of mass-to-charge ratios from 350 to 3000. Accuracy, precision, selectivity, sensitivity, reproducibility, and stability using this technique are described in detail elsewhere.[16,17] In brief, the detection limit is in the range of 1 fmol, depending on the ionization properties of the individual peptide. In a urine sample, the detection limit in the crude sample before processing is 100 to 1000 fmol/mL. Platform validation was performed as described previously.[18]

Data Processing

Data were analyzed using the Mosaiques-Visu software;[19] CE/MS peaks were detected using a signal-to-noise ratio of 4, and the charge of each peak was calculated based on isotopic distributions and conjugated masses. The data were then deconvoluted, allowing mass spectral ion peaks from the same molecule at different charge states to be recorded as a single mass.

MS data were normalized to correct for analytic issues such as signal suppression as well as biological issues such as hydration of the patient. Reference signals of >1700 urinary peptides were used for CE time calibration.[16] MS signal intensities were normalized relative to 29 "housekeeping" peptides with small relative standard deviation. These peptides, which are the result of normal biological processes, are not affected by age, sex, or disease state. Normalization using these peptides has been shown to be as accurate as absolute peptide quantification using urinary quantification or stable isotope-labeled synthetic marker analogues.[17] For time-of-flight MS mass calibration, 80 reference masses exactly determined by Fourier transform ion cyclotron resonance mass spectrometry were used. The resulting peak list characterizes each peptide by its molecular mass (Da), CE migration time, and signal intensity, providing a unique identification mark. Data were entered into a Microsoft SQL database for further analysis and comparison with other samples. MS peaks from different samples were presumed identical if mass deviation was ≤50 ppm for small or ≤75 ppm for larger peptides, and the migration time deviation was <5 minutes.

Disease-specific polypeptide patterns were generated using support vector machine-based MosaCluster software[20] as described recently.[21] In brief, MosaCluster displays data points (urine samples) from "n" proteomic markers as an "n"-dimensional vector and attempts to separate them by (n−1) dimensional hyperplane. A classification factor was calculated for each sample from the distance and direction of its vector to the separating hyperplane.

Sequencing

Candidate biomarkers were sequenced using liquid chromatography-tandem mass spectrometry analysis as described,[11,22] including the use of instruments with electron transfer dissociation capability.[23–25] Spectral data were searched against the SwissProt database using the open MS search algorithm (http://pubchem.ncbi.nlm.nih.gov/omssa/; accessed December 21, 2010) using an e-value cut-off of 1.00×10^{-2}. All matched sequences were manually validated. All sequences obtained from human urine can be assessed at http://mosaiques-diagnostics.de/diapatpcms/mosaiquescms/front_content.php?idcat=257 (accessed December 21, 2010).[26]

Statistical Methods, Definition of Biomarkers, and Sample Classification

Estimates of sensitivity and specificity were calculated based on tabulating the number of correctly classified samples. Confidence intervals (95% CI) were based on exact binomial calculations and were performed in MedCalc version 8.1.1.0 (MedCalc Software). The reported unadjusted probability values were calculated using the natural logarithm-transformed intensities and the Wilcoxon rank-sum test. Statistical adjustment for multiple testing was performed either using the stringent max*t* test[27] or using false discovery rate adjustments of Benjamini and Hochberg.[28]

Results

Urinary Proteome of Healthy Pregnancy

We initially investigated whether differences exist in the urinary proteome of healthy pregnant women (who did not subsequently have preeclampsia develop; n=17) when compared to apparently healthy, nonpregnant, female volunteers (n=67). Clear differences between these 2 cohorts were seen (Figure 1). On examination of the datasets, 284 peptides that were significantly altered between the 2 groups were identified by using the max*t* test. The discriminatory peptides are listed in Supplemental Table S4 (please see http://hyper.ahajournals.org).

The pregnancy-associated urinary peptides were then evaluated in a second set of independent samples: 248 urine samples from pregnant women that were included in the study and 141 apparently healthy nonpregnant controls. Support vector machine classification based on the 284 peptides revealed 98.6% specificity and 98.4% sensitivity in the independent dataset.

Biomarkers for Prediction of Preeclampsia at Gestational Week 28

Our next step was to investigate whether biomarkers for the prediction of preeclampsia could be detected in the urinary proteome at gestational week 28. Among the women with at least 2 risk factors for preeclampsia, 18 samples from women who subsequently had preeclampsia develop (cases) and 17 samples from women who had normotensive pregnancies (controls) were examined (Figure 2). After adjustment for multiple testing (Benjamini-Hochberg), 10 biomarkers that were significantly associated with preeclampsia were identified. A model containing these 10 biomarkers differentiated between cases and controls ($P<0.001$). We then constructed a biomarker model that included an additional 40 peptides that were nominally significant between cases and controls and had highest significance scores in the absence of false discovery rate adjustment. This more stable model enabled classification of the samples with 100% sensitivity and specificity, even when evaluated using complete take-one-out cross-validation. Classification factor was 1.032±0.411 in women who subsequently had preeclampsia develop compared to −1.038±0.432 ($P<0.001$) in those whose pregnancy continued without preeclampsia developing (Figure 3). Details of the biomarkers, which include sequences of collagen chains, fibrinogen, and uromodulin, used in the model are shown in Table 2.

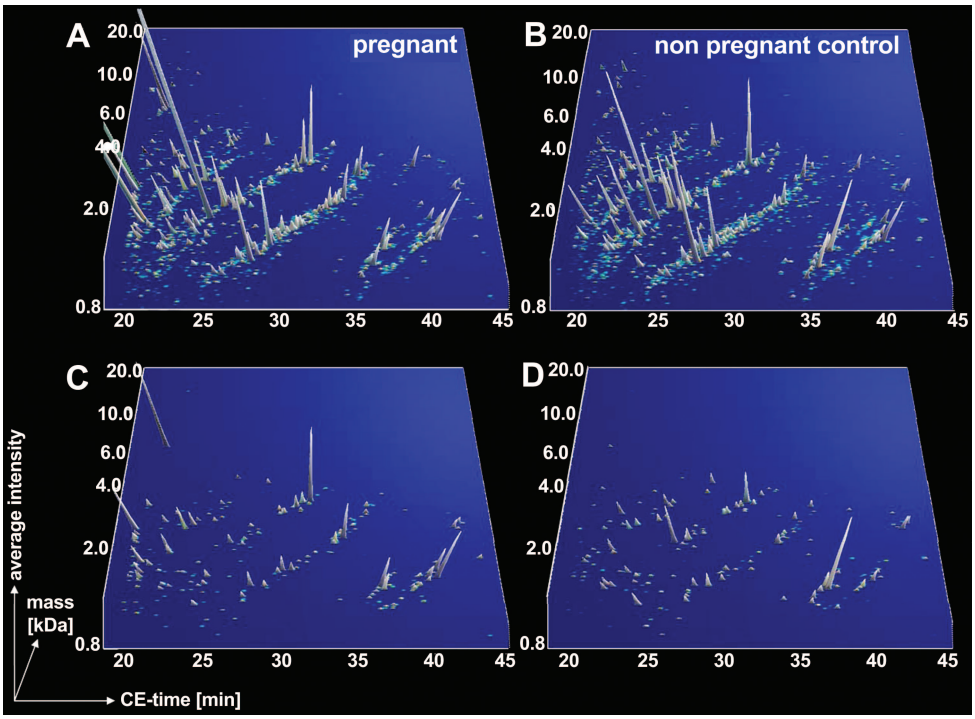

Figure 1. Pregnancy-specific urinary polypeptide signatures. Capillary electrophoresis coupled to mass spectrometry profiling of urine (top, A and B) resulted in the definition of 284 polypeptides defining a pregnancy-specific polypeptide signature of urine (bottom, C and D). Normalized molecular weight (800–20 000 Da) in logarithmic scale is plotted against normalized migration time (18–45 minutes). The mean signal intensity of the polypeptide peak is given in 3-dimensional depiction. Compiled data sets of 17 pregnant (A and C) and 67 nonpregnant women (B and D) are shown.

Biomarkers for Prediction of Preeclampsia in the First or Second Trimester

We then investigated whether this 50-marker biomarker panel could be applied to samples from earlier in pregnancy to predict preeclampsia. In a first step, we analyzed in the PIP study samples from gestational week 12 to 16. Urine samples from 45 women who subsequently had preeclampsia develop were matched for age and body mass index to 86 controls

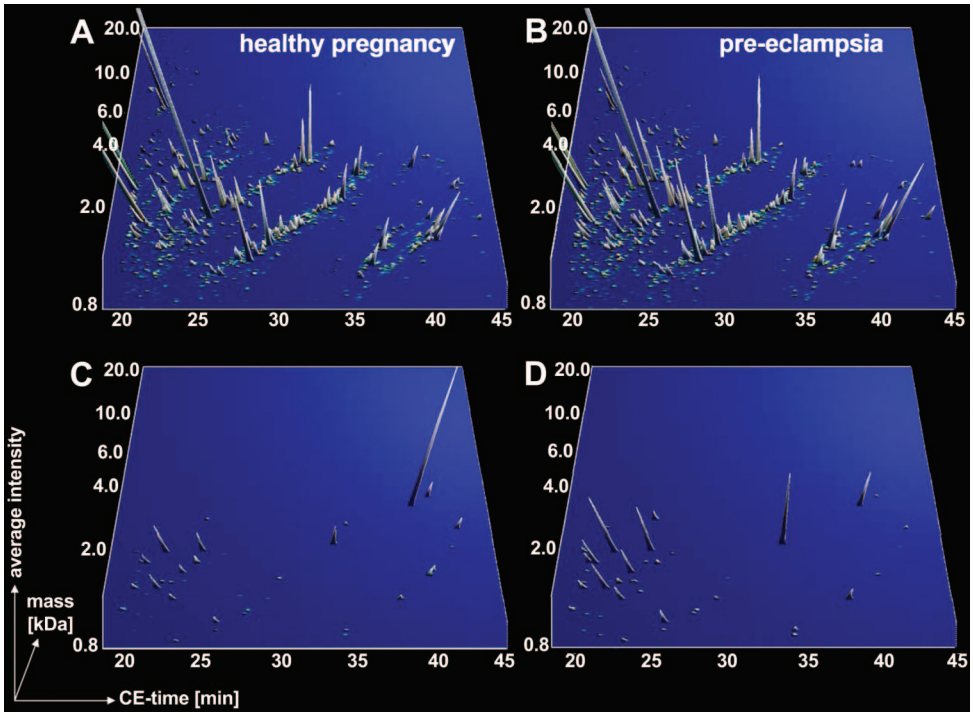

Figure 2. Urinary biomarkers that predict preeclampsia. Urine samples from gestational week 28 from women who had normotensive pregnancies (n=17; A and C) and who subsequently had preeclampsia develop (n=18; B and D) were analyzed. A urinary polypeptide signature of 50 pregnancy-specific biomarkers was defined (bottom, C and D). Technical details are similar to those of Figure 1.

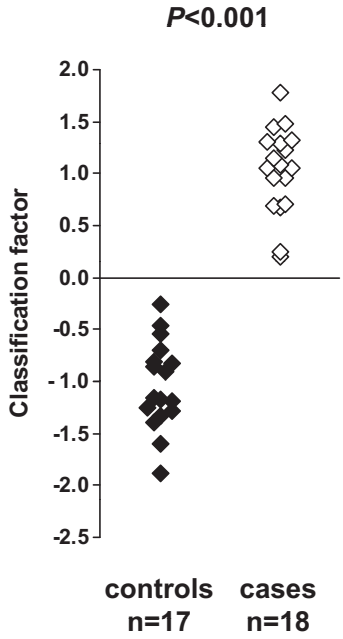

Figure 3. Classification of preeclampsia-specific urinary biomarkers. Translation of the preeclampsia-specific urinary polypeptide signature (Figure 2) into a classification factor demonstrates significant difference between women at gestational week 28 who subsequently had preeclampsia develop (cases; open diamonds) and women who had normotensive pregnancies (controls; filled diamonds). Sensitivity and specificity were 100%, with positive numbers predicting high risk for preeclampsia.

with normal pregnancies. Using the preeclampsia pattern generated from week 28, we were able to differentiate between those who went on to have preeclampsia develop (classification factor, -0.567 ± 0.372) and those who had uncomplicated pregnancies (classification factor, -0.721 ± 0.439; $P=0.047$; Figure 4A). In a second step, we compared the classification factors in women with known risk factors who had provided urine samples both at weeks 12 to 16 and week 28. Classification factor decreased slightly from gestational week 12 to 16 to 28 in those women who were sampled at both times and had a normal pregnancy (n=16; from -0.647 ± 0.437 to -1.024 ± 0.433; $P=0.043$; Figure 5A), whereas it markedly changed toward positive scores in those women who subsequently had preeclampsia develop (n=16; from -0.392 ± 0.383 to 1.070 ± 0.383; $P<0.001$; Figure 5B). In a third step, we analyzed urine samples from gestational week 20 of 99 women (50 cases, 49 controls) from the SCOPE study. In this cohort, there was no significant difference between cases (classification factor, -0.755 ± 0.533) and controls (-0.724 ± 0.418; $P=0.73$; Figure 4B), suggesting that the biomarkers identified at week 28 do not reliably predict preeclampsia when analyzed earlier in pregnancy. Finally, the samples from gestational week 12 to 16 and 20 were analyzed to find whether other biomarker patterns could be used to accurately differentiate between cases and controls, and thereby to predict preeclampsia, at an earlier time in pregnancy. We were unable to identify any such early predictive biomarker patterns.

Discussion

We designed a large study (PIP) in which we used CE-MS technology to examine the urinary proteome in pregnancy and attempted to identify a urinary proteomic signature that can be used in early and mid pregnancy to predict preeclampsia. At gestational week 28, we were able to identify a urinary peptide pattern, characterized by breakdown products of fibrinogen, collagen, and uromodulin, which could differentiate women who subsequently had preeclampsia develop. Although there is a limited role for late screening for preeclampsia, this set of biomarkers may prove to be a helpful aid in diagnosis of preeclampsia. We were able to use this peptide pattern to differentiate between cases and controls at gestational week 12 to 16, but we were not able to replicate this in an independent cohort of pregnant women (SCOPE) at gestational week 20. The magnitude of overlap between cases and controls at 12 to 16 weeks and failure to replicate our findings in another cohort precludes use of this urinary peptide pattern as an early screening test. The different findings in the 2 cohorts may result from differences in gestational age at sampling (20 weeks rather than 28 weeks) and study populations; SCOPE comprised healthy nulliparous women, whereas PIP was a mixed-parity general obstetric population.

The performance of our urinary peptide pattern at 28 weeks to discriminate women who subsequently had preeclampsia develop from those with an uncomplicated pregnancy is similar or better than that reported with serum FMS-like tyrosine kinase-1 and placental growth factor in the second trimester for early-onset preeclampsia and is a better predictor than these markers for all preeclampsia (preterm and term disease).[29–31] The discrimination seen with the urinary peptide pattern at 28 weeks is more aligned with the diagnostic performance of the angiogenic biomarkers after presentation with symptoms or signs of preeclampsia.[32,33] Because our study is small, it requires further evaluation in larger predictive and diagnostic studies to define its potential role in clinical care.

To date, 1 other study has used a proteomic technique to identify disease-specific urinary biomarkers for preeclampsia after diagnosis. Buhimschi et al[7] identified fragments of serpin peptidase inhibitor-1 and albumin as biomarkers for preeclampsia, and for differentiation of preeclampsia from other hypertensive disorders of pregnancy. Increased urinary albumin excretion is a hallmark of preeclampsia and serpin peptidase inhibitor-1 increases >10-fold in pregnancy (unpublished observations, R. North). We would expect our findings to be different because they used SELDI technology to investigate the urinary proteome.

The differentially expressed polypeptide sequences between cases and controls at gestational week 28 point toward biological processes that are altered during the development of preeclampsia, particularly the late-onset variant of the disease. Changes in extracellular matrix are a feature of all vascular diseases and are represented by a number of differentially expressed collagen fragments in the polypeptide signatures. These findings are in line not only with plasma proteome data by Blumenstein et al[34] but also with our previous studies of urinary proteomic markers of chronic

Table 2. Preeclampsia-Specific Proteomic Biomarkers

Mass (Da)	CE Time (min)	Unadjusted *P*	Sequence	Protein Name	Start Amino Acid	Stop Amino Acid	Swissprot Name	Accession N
3657.665	**40.71**	**3.04E-04**						
3292.541	39.42	3.29E-03						
2841.256	24.54	2.17E-03	ERGEAGlpGVpGAkGEDGKDGSpGEpGANG	Collagen alpha-1 (III) chain	448	477	CO3A1_HUMAN	gi124056490
2674.217	34.58	1.34E-03						
2658.271	19.48	4.59E-04	DEAGSEADHEGTHSTKRGHAKSRPV	Fibrinogen alpha chain	605	629	FIBA_HUMAN	gi1706799
2587.195	21.10	3.21E-03						
2570.190	42.56	2.43E-03						
2196.993	33.69	1.03E-03						
2117.032	42.00	2.35E-03						
2048.927	**24.46**	**9.53E-05**						
2030.912	21.85	1.80E-03	EGSpGRDGSpGAkGDRGETGP	Collagen alpha-1 (I) chain	1021	1041	CO1A1_HUMAN	gi124056487
2019.876	19.75	1.54E-03						
2014.898	**21.91**	**4.69E-05**	**EGSpGRDGSpGAKGDRGETGP**	**Collagen alpha-1 (I) chain**	**1021**	**1041**	**CO1A1_HUMAN**	**gi124056487**
1968.900	**25.96**	**4.45E-05**	**ASTRESGVPDRFSGSGSGTD**	**Ig kappa chain V-IV region B17**	**77**	**96**	**KV404_HUMAN**	**gi125834**
1817.694	20.23	8.33E-04						
1812.786	24.14	1.87E-03						
1807.809	20.65	1.81E-03	SVDETGQmSATAKGRVR	Retinol-binding protein 4	64	80	RET4_HUMAN	gi62298174
1795.793	**25.00**	**8.73E-06**						
1668.805	40.47	5.44E-04						
1652.698	20.13	8.53E-04						
1640.581	23.24	1.01E-03						
1594.762	40.22	2.09E-03	pGpSGLPGLPGpPGPPGP	Collagen alpha-3 (IX) chain	141	158	CO9A3_HUMAN	gi20137327
1540.772	29.87	2.21E-03	SGSVIDQSRVLNLGP	Uromodulin	589	603	UROM_HUMAN	gi137116
1495.684	**23.36**	**2.35E-04**						
1484.666	23.57	2.46E-03						
1482.666	22.47	1.80E-03						
1474.658	20.05	1.68E-03						
1417.635	**20.03**	**2.55E-04**						
1407.602	21.61	1.24E-03						
1405.635	20.14	2.78E-03	DGPpGRDGQpGHKG	Collagen alpha-2 (I) chain	933	946	CO1A2_HUMAN	gi124056488
1353.532	23.96	1.10E-03						
1319.584	20.89	3.16E-03						
1294.601	27.24	3.16E-03	SpGNIGPAGKEGPV	Collagen alpha-2 (I) chain	455	468	CO1A2_HUMAN	gi124056488
1270.503	38.07	2.26E-03						
1268.565	29.11	1.58E-03						
1263.543	22.73	1.29E-03						
1154.576	19.53	2.01E-03						
1138.586	19.51	3.79E-03						
1135.490	27.82	3.22E-03						
1101.537	27.62	1.18E-03	FRFAGNYDL	Uromodulin	553	561	UROM_HUMAN	gi137116
1091.482	20.51	2.64E-03						
1071.494	21.43	2.70E-03						
1016.445	25.79	9.50E-04	ApGDKGESGPS	Collagen alpha-1 (I) chain	777	787	CO1A1_HUMAN	gi124056487
949.219	**34.33**	**8.00E-05**						
945.416	25.72	2.00E-03						
942.449	20.46	3.21E-03						
906.179	34.26	9.07E-04						
903.410	21.58	1.07E-05						
858.393	**23.24**	**1.02E-03**	**SpGEAGRpG**	**Collagen alpha-1 (I) chain**	**522**	**530**	**CO1A1_HUMAN**	**gi124056487**
806.305	**22.67**	**2.83E-05**						

Biomarkers that differentiate between women who subsequently have preeclampsia develop and those with normal pregnancy are listed. Sequence information and protein identifiers are given where available. Unadjusted *P* are from Wilcoxon nonparametric test. Ten markers that remained significantly different after adjustment for multiple testing are in bold.

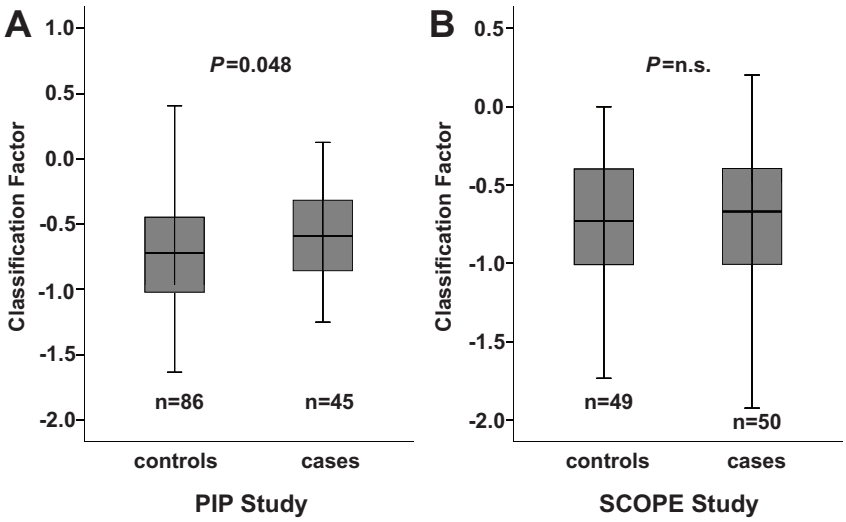

Figure 4. Earlier prediction of preeclampsia. The classification factor was not different between controls and women with future preeclampsia at gestational week 12 to 16 (PIP study; A) and at gestational week 20 (SCOPE study; B), indicating no consistently reproducible difference in urinary polypeptide expression between these groups in the late first and early second trimesters.

kidney disease[11,17] and coronary artery disease.[9,10,21] These conditions share some of the features of preeclampsia, including endothelial dysfunction and inflammation,[35] and therefore may also share some of the characteristic proteomic markers with preeclampsia.

Differential expression of uromodulin sequences in urine between cases and controls is not surprising. Uromodulin (Tamm-Horsfall protein) is the most abundant protein in normal urine; therefore, this could simply indicate nonspecific changes in the composition of urine. The *UMOD* gene locus, however, has been found to be associated with glomerular filtration rate in genetic studies,[36] indicating a potential functional role of uromodulin in the development of cardiovascular diseases. More recently, we have demonstrated in a genome-wide association study that rs13333226 in the promoter region of *UMOD* is associated with hypertension independently of renal function.[37] These more recent data on uromodulin, as a key player in renal disease and hypertension, could point toward subclinical vascular and renal damage in the early stage of preeclampsia that are indicated by differential urinary expression of uromodulin fragments and warrant further investigation.

It should also be noted that in our approach, we restricted the analysis to peptides and proteins with a molecular mass >800 Da that, in addition, had to be at least 2-fold different between cases and controls. Whereas typical pregnancy-associated hormones such as estrogen or progesterone and their respective metabolites generally can be detected in urine using mass spectrometry,[38] the sample preparation and the data evaluation used in our study essentially excluded these and most other metabolites because of their smaller molecular mass and the generally observed single charge of metabolites. Our analytic approach therefore should be regarded as complementary to other approaches for the identification of diagnostic and predictive markers including metabolomic studies.[39]

Perspectives

The pathogenesis of preeclampsia is thought to begin in the first trimester of pregnancy and is characterized by impaired placental implantation and development. Detection of the pathophysiological changes in the first trimester would be a key to early detection of the disease and would be extremely useful for early risk stratification. The fact that we were unable to reliably detect preeclampsia before gestational week 28 and the nature of the biomarkers sequenced suggest that we were able to detect early signs of preeclampsia rather than the underlying causes.

In clinical practice, proteinuria is used to differentiate gestational hypertension from preeclampsia. Current tests for proteinuria have a number of limitations,[40] and a better measure of renal involvement in preeclampsia is required to improve accurate diagnosis. Proangiogenic and antiangiogenic factors (placental growth factor and serum FMS-like

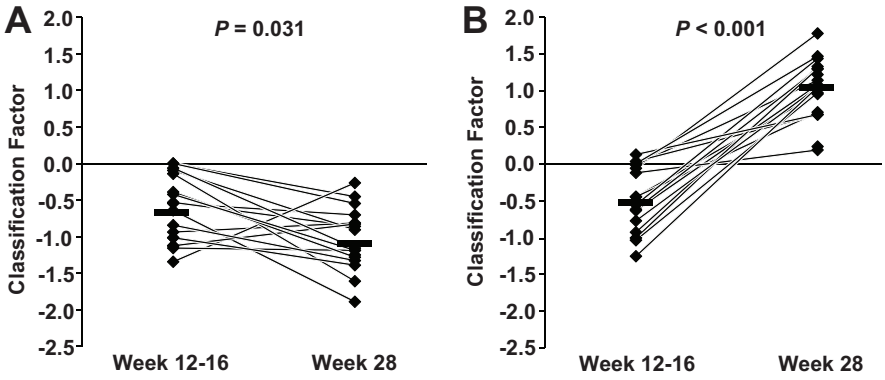

Figure 5. Change in classification factor during pregnancy. A, Changes in classification factor from gestational week 12 to 16 to 28 in normotensive pregnancy (n=16; A) and in women who subsequently had preeclampsia develop (n=16; B). Horizontal lines indicate mean values.

tyrosine kinase-1) are being investigated as potential diagnostic tests for preeclampsia, especially early-onset disease, but their diagnostic utility in term preeclampsia is much less certain.[33] The biomarkers we detected may have a role in improving the diagnosis of women with late-onset preeclampsia, whereas their potential to act as a predictive test and to trigger preventative therapy and more intensive monitoring may be limited.

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Disclosures

H.M. is founder and director of Mosaiques Diagnostics GmbH, which develops urinary proteomics for use in clinical practice. J.S., P.Z., and J.F. are employees of Mosaiques Diagnostics GmbH. The other authors report no conflict of interest.

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ONLINE SUPPLEMENT

URINARY PROTEOMICS FOR PREDICTION OF PRE-ECLAMPSIA

David M Carty [1], Justyna Siwy [2], Janet E Brennand [3], Petra Zürbig [2], William Mullen [4], Julia Franke [2], James W McCulloch [1], Robyn A North [5], Lucy C Chappell [5], Harald Mischak [1,2], Lucilla Poston [5], Anna F Dominiczak [1], and Christian Delles [1]

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Supplementary Table S1. Characteristics of the biomarker discovery cohort (gestational week 28).

Characteristic	Preeclampsia n=18	Controls n=17	*P*-value
Maternal Characteristics			
Age (years)	30±4	29±4	0.62
Ethnicity			
Caucasian	16 (89%)	17 (100%)	0.49
Other	2 (11%)	0 (0%)	
Body mass index (kg/m^2)	30±5	30±4	0.75
Nulliparous	16 (89%)	9 (53%)	0.03
Smoker	1 (6%)	4 (24%)	0.18
Previous pre-eclampsia	1 (6%)	5 (29%)	0.09
Family history of pre-eclampsia (mother or sister)	8 (44%)	4 (24%)	0.29
At Week 28			
Systolic blood pressure (mmHg)	130±10	126±9	0.17
Diastolic blood pressure (mmHg)	80±8	73±6	0.01
Proteinuria (≥+ on dipstick)	2 (11%)	1 (6%)	1.00
Gestation at sampling (wks)	28.2±1.4	28.6±1.5	0.37
Pregnancy Outcome			
Highest systolic blood pressure (mmHg)	159±9	126±10	<0.001
Highest diastolic blood pressure (mmHg)	100±6	73±7	<0.001
Caesarean section	4 (22%)	3 (17%)	1.00
Gestation at delivery (weeks)	39.3±2	40.5±1	0.02
Birthweight (g)	3277±419	3722±468	0.001
Customised birthweight centile	36 [11;49]	47 [37;57]	0.16
Small for gestational age (<10th customised birthweight centile)	4 (22%)	0 (0%)	0.10

All data are mean ± standard deviation, median [interquartile range] or number (percent of total) from the study visit at gestational week 28 Comparisons between cases and controls were made using Student's t-test or Fisher's exact test as appropriate.

Supplementary Table S2. Characteristics of the biomarker validation cohort (PIP study, gestational week 12-16).

Characteristic	Preeclampsia n=45	Controls n=86	*P*-value
Maternal Characteristics			
Age (years)	29±5	28±5	0.69
Ethnicity			
Caucasian	42 (93%)	81 (94%)	1.00
Other	3 (7%)	5 (6%)	
Body mass index (kg/m^2)	29±5	29±5	0.76
Nulliparous	39 (87%)	55 (64%)	0.01
Smoker	4 (9%)	21 (24%)	0.04
Previous pre-eclampsia	1 (2%)	6 (7%)	0.42
Family history of pre-eclampsia (mother or sister)	8 (17%)	7 (8%)	0.14
At Booking			
Systolic blood pressure (mmHg)	121±10	114±12	0.002
Diastolic blood pressure (mmHg)	76±9	69±10	0.001
Proteinuria (≥+ on dipstick)	1 (2%)	2 (2%)	1.00
Gestation at sampling (wks)	13.8±1.6	13.7±1.3	0.61
Pregnancy Outcome			
Highest systolic blood pressure (mmHg)	165±11	125±12	<0.001
Highest diastolic blood pressure (mmHg)	101±8	71±9	<0.001
Caesarean section	20 (44%)	10 (12%)	<0.001
Gestation at delivery (wks)	38.3±2	40±1.4	<0.001
Birthweight (g)	3155±621	3558±416	<0.001
Customised birthweight centile	37 [10;61)	48 [33;67]	0.09
Small for gestational age (<10th customised birthweight centile)	12 (27%)	3 (3%)	<0.001

All data are mean ± standard deviation, median [interquartile range] or number (percent of total). Comparisons between cases and controls were made using Student's t-test or Fisher's exact test as appropriate.

Supplementary Table S3. Characteristics of the biomarker validation cohort (SCOPE study, gestational week 20).

Characteristic	Preeclampsia n=50	Controls n=49	*P*-value
Maternal Characteristics			
Age (years)	26±6	26±6	0.93
Ethnicity			
Caucasian	41 (82%)	42 (86%)	0.62
Other	9 (18%)	7 (14%)	
Body mass index (kg/m^2)	27±6	25±6	0.12
Gravidity			
1	42 (84%)	37 (76%)	0.29
≥2	8 (16%)	12 (24%)	
Smoker	5 (10%)	7 (14%)	0.51
At 20 weeks gestation			
Systolic blood pressure (mmHg)	114±10	109±10	0.01
Diastolic blood pressure (mmHg)	68±10	64±7	0.05
Gestation at blood sampling (wks)	20.1±0.7	20.1±0.6	0.86
Pregnancy Outcome			
Systolic blood pressure (mmHg)	148±15	118±10	<0.001
Diastolic blood pressure (mmHg)	95±9	70±9	<0.001
Caesarean section	18 (36%)	9 (18%)	0.05
Gestation at delivery (wks)	38.5±1.6	40.3±1.0	<0.001
Birthweight (g)	3090±616	3609±407	<0.001
Customized birthweight centile	40 [18;66]	55 [40;74]	0.02
Small for gestational age (<10th customized birthweight centile)	9 (18%)	0 (0%)	0.003

All data are mean ± standard deviation, median [interquartile range] or number (percent of total). Comparisons between cases and controls were made using Student's t-test or Fisher's exact test as appropriate.

Correction

In the *Hypertension* article by Carty et al (Carty DM, Siwy J, Brennand JE, Zürbig P, Mullen W, Franke J, McCulloch JW, North RA, Chappell LC, Mischak H, Poston L, Dominiczak AF, Delles C. Urinary Proteomics for Prediction of Preeclampsia. *Hypertension*. 2011;57[part 2]:561-569), an author's name was erroneously omitted from the author line. Claire T. Roberts's name is to be added to the list of authors.

The corrected author line and affiliations should be as follows:

David M. Carty, Justyna Siwy, Janet E. Brennand, Petra Zürbig, William Mullen, Julia Franke, James W. McCulloch, Claire T. Roberts, Robyn A. North, Lucy C. Chappell, Harald Mischak, Lucilla Poston, Anna F. Dominiczak, Christian Delles.

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The authors regret this omission.