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Applicability of a high-throughput shotgun plasma protein screening approach in understanding maternal biological pathways relevant to infant birth weight outcome☆,☆☆

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ABSTRACT

There are reports linking maternal nutritional status, smoking and environmental chemical exposures to adverse pregnancy outcomes. However, biological bases for association between some of these factors and birth outcomes are yet to be established. The objective of this preliminary work is to test the capability of a new high-throughput shotgun plasma proteomic screening in identifying maternal changes relevant to pregnancy outcome. A subset of third trimester plasma samples (N = 12) associated with normal and low-birth weight infants were fractionated, tryptic-digested and analyzed for global proteomic changes using a MALDI-TOF–TOF-MS methodology. Mass spectral data were mined for candidate biomarkers using bioinformatic and statistical tools. Maternal plasma profiles of cytokines (e.g. IL8, TNF-α), chemokines (e.g. MCP-1) and cardiovascular endpoints (e.g. ET-1, MMP-9) were analyzed by a targeted approach using multiplex protein array and HPLC-Fluorescence methods. Target and global plasma proteomic markers were used to identify protein interaction networks and maternal biological pathways relevant to low infant birth weight. Our results exhibited the potential to discriminate specific maternal physiologies relevant to risk of adverse birth outcomes. This proteomic approach can be valuable in understanding the impacts of maternal factors such as environmental contaminant exposures and nutrition on birth outcomes in future work.

Biological significance
We demonstrate here the fitness of mass spectrometry-based shot-gun proteomics for surveillance of biological changes in mothers, and for adverse pathway analysis in

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combination with target biomarker information. This approach has potential for enabling early detection of mothers at risk for low infant birth weight and preterm birth, and thus early intervention for mitigation and prevention of adverse pregnancy outcomes.
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1. Introduction

There is rising concern due to association between pregnancy outcomes and onset of childhood and adult diseases [1,2]. Perinatal health outcomes are important markers of future child and adult health [3]. Maternal determinants of adverse pregnancy outcomes can include maternal nutrition, age, smoking, disease, and environmental contaminant exposure levels [4–9]. For instance, there are reports on maternal nutrition in different stages of pregnancy affecting placental and infant size [10] as well as preterm birth and fetal growth retardation [11]. Similarly, diet in pregnancy has been shown to affect offspring's blood pressure in their early adulthood [12]. Previous works have shown that both extremely young and advanced maternal ages can lead to poor pregnancy outcomes [13,14]. Maternal smoking has been implicated in miscarriage, perinatal mortality, birth defects, low birth weight and premature births [15,16]. Also, heart disease has been associated with maternal and neonatal complications in pregnancy [17]. Exposures to diesel exhausts during pregnancy can lead to pathologies similar to autism in infants [18]. There are also studies suggesting pregnancy time-dependant vulnerability to different components of air pollutants [19,20].

Mechanistic understanding of the maternal biological pathways which play a role in adverse pregnancy outcome is critical in terms of managing the risk during pregnancy. Elevated circulating endothelin-1 (ET-1) levels and high blood pressure in pregnant women are associated with intrauterine growth restriction (IUGR) resulting in low infant birth weights [21]. Similarly, oxidative stress has been reported to cause maternal and fetal morbidities [22–26], and is implicated as a major factor in preeclampsia [27]. Molecular mechanisms by which maternal factors influence fetal development are still poorly characterized.

Traditionally, most of the methodologies that are used to understand maternal biological mechanisms involved in poor pregnancy outcome are based on target endpoint analyses, a reductionist approach [28]. Proteomic analyses exhibit a greater potential in viewing changes at a global level. One such approach is shotgun proteomic analysis [29,30]. This refers to rapid and direct analysis of multiple proteins simultaneously in a protein mixture permitting qualitative and quantitative assessment of their changes in biological systems. There are various methodologies employed to conduct shotgun proteomic analyses including analyses based on two-dimensional gel electrophoresis separation followed by mass spectrometry by both MALDI-TOF–TOF-MS and ESI-MS/MS platforms, multidimensional LC based separations followed by tandem mass spectrometry. However, there are reports on limitations associated with global analysis of proteins when applied to real biological systems [31].

Our objective was to test the ability of a simple high-throughput shotgun plasma proteomic screening approach to discriminate between maternal physiologies relevant to different types of pregnancy outcomes. For this purpose, we used a very small subset of third trimester plasma samples from a mother–infant cohort (Maternal-infant Research on Environmental Chemicals-(MIREC) Study). Plasma proteomic changes were assessed by a global MS-based proteomic analysis method and the m/z data was used for protein profiling. Meanwhile, an array of target protein markers were analyzed in a second set of matched 3rd trimester maternal plasma samples. Information on these target marker levels and candidate protein marker results obtained by the global proteomic method were used for exploring maternal mechanisms relevant to low infant birth weight outcome.

2. Methods

2.1. Materials

Dulbecco's phosphate-buffered saline (PBS, calcium and magnesium free), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DETPA), phenylmethylsulfonyl fluoride (PMSF), trifluoroacetic acid (TFA), 3,4-dichloroisocoumarin, molecular weight cut-off filters (30, 50 and 100 kDa) and endothelin isoform standards for the HPLC-Fluorescence analyses namely, endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3) were purchased from Sigma (St. Louis, MO, USA). The big endothelin-1 (BET-1) isoform was from Bachem Americas (Torrance, CA, USA). Reagent-grade acetone, acetonitrile, and methanol were from ThermoFisher (Ottawa, ON, Canada). Butylatedhydroxytoluene (BHT) was from United States Biochemical Corporation (Cleveland, OH, USA). Deionzed water (DI water) was obtained from a super-Q plus high purity water system (Millipore, Bedford, MA, USA). UHP-grade compressed nitrogen was supplied by Matheson Gas products (Whitby, ON, Canada). Amber glass vials and screw caps with septa were purchased from Chromatographic Specialties Inc. (Brockville, ON, Canada). Antiprotease (Halt protease inhibitor) cocktail was obtained from ThermoFisher (Ottawa, ON, Canada). Peptide/protein calibration standards and the matrix α-cyano-4-hydroxy cinnamic acid were purchased from Bruker Daltonics (Bremen, Germany). Sequence grade trypsin was obtained from Promega Corporations (Madison, WI, USA). Bioplex kits were purchased from either Millipore (Billerica, MA, USA) or BioRad (Mississauga, ON, Canada).

2.2. Maternal plasma samples associated with low and healthy birth weight infants

Third trimester maternal plasma samples were obtained from the MIREC study cohort described by Arbuckle et al. [32]. A

very small subset of samples (N = 12/group) of cases and controls (mothers associated with infants of low (<2700 g) and normal (2700–4300 g) birth weights) were used in this study since this is an exploratory high throughput shotgun proteomic analysis undertaken to determine its use in future screening of maternal samples for adverse outcome pathways. For the purpose of this analysis and to increase our study power, low birth weight was defined as less than the 10th percentile of all birth weights in the cohort (i.e. <2700 g). Infant birth weights and gestational ages were abstracted from the medical charts at delivery. Systolic and diastolic blood pressure values were measured during the third trimester clinic visit when the blood samples were collected.

2.3. Ethics

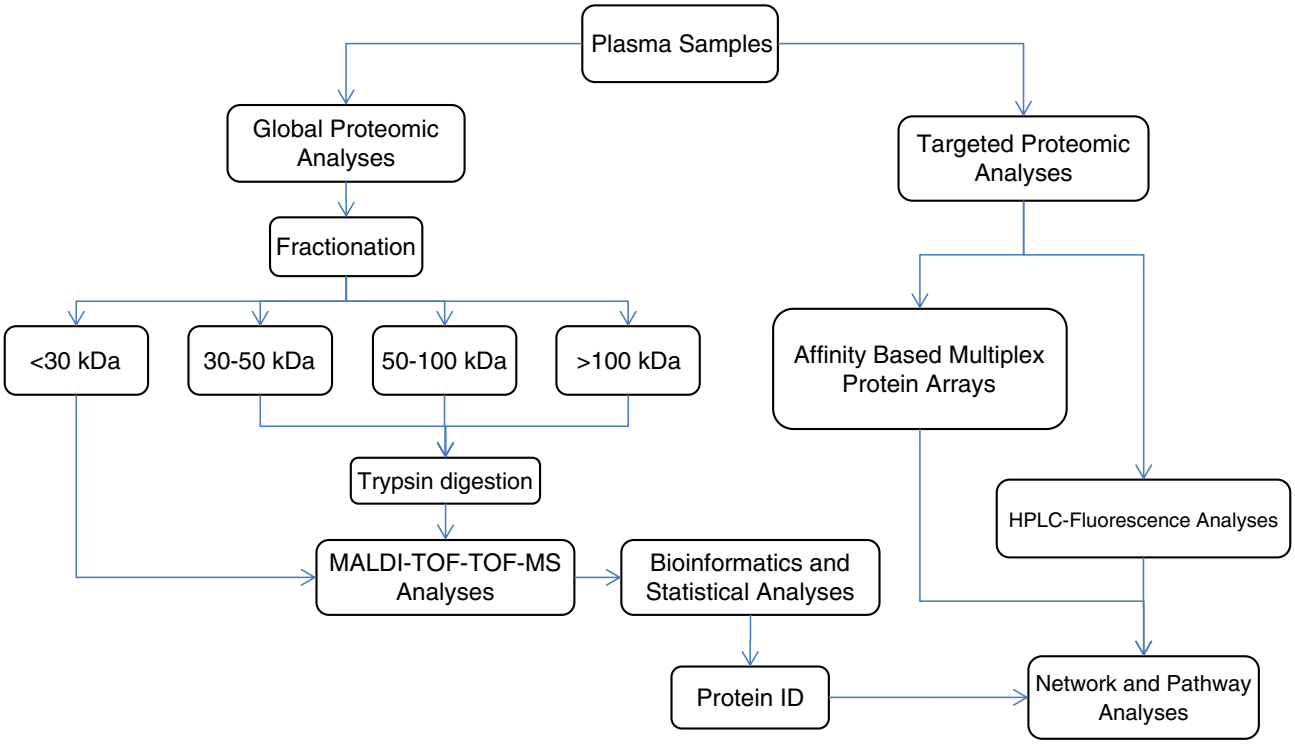
The details of the ethics review of the MIREC study are described by Arbuckle et al. [32]. Briefly, the research protocol, questionnaires, consent forms and recruitment posters and pamphlets were reviewed and approved by human studies research ethics committees, including the Research Ethics Board at Health Canada and the ethics committee at the coordinating center at St-Justine's Hospital in Montreal, as well as more than ten academic and hospital ethics committees across Canada.

2.4. Plasma sample preparation

Aliquots of plasma samples derived from the 3rd trimester maternal whole blood samples (N = 12/group, low vs. normal infant birth weight groups) stabilized with preservatives (EDTA, PMSF) [33], were treated with DETPA, BHT and antiprotease cocktail, were vortexed, and frozen for storage. Matching sets of aliquots of plasma were analyzed for target biomarkers namely, circulating vasoregulatory peptides (endothelins), inflammatory cytokines, chemokines, other cardiovascular markers including acute phase proteins, and for global biomarkers using a MS-based shotgun proteomic analysis method (Scheme 1).

2.5. Sample preparation for global proteomic analysis

The plasma samples for global proteomic biomarker analysis were fractionated using molecular weight cut-off filters (Millipore, Billerica, MA, USA) to obtain <30 kDa, 30–50 kDa and 50–100 kDa fractions as described previously by Kumarathasan et al., 2012 [34]. Briefly, 60 μL aliquots of plasma samples were thawed on ice, diluted with 200 μL of deionized H_2O, and were vortexed. Molecular weight cut-off (MWCO) filters (30 kDa) were pre-wetted by passing 200 μL deionized water at 14,000 ×*g* for 10 min. The filtrate was discarded and the above diluted plasma samples were transferred into the pre-wetted MWCOs. The content was centrifuged at 14,000 ×*g* for 10 min. The filtrates (<30 kDa fractions) were collected, and the residues were diluted with 200 μL deionized H_2O, vortexed gently and the filter units were inverted and the residue samples were collected into fresh clean tube by centrifugation at 14,000 ×*g* for 10 min. The filter unit was washed with additional 75 μL of deionized H_2O, vortexed gently, inverted and centrifuged again to transfer the wash into the same collection tube. These >30 kDa residue samples were transferred into a pre-wetted 50 kDa MWCO filters and centrifuged at 14,000 ×*g* for 10 min. The filtrates (30–50 kDa) were collected, and the residues from this step were subjected to the process above to obtain the 50–100 kDa and the subsequent



Scheme 1 – Work flow for sample preparation, analyses and data processing.

>100 kDa fractions using the appropriate pre-wetted MWCO filters. The plasma fractions other than the <30 kDa were digested with trypsin following the procedure reported earlier [34,35]. <30 kDa fractions were saved for endogenous peptides analyses. All tryptic digested and non-digested fractions of the plasma samples were evaporated under a flow of nitrogen, and were stored at −80 °C prior to being analyzed.

2.6. MALDI-TOF–TOF-MS analysis of plasma samples

Frozen, dry tryptic-digested and non-digested plasma fractions were thawed at room temperature and were reconstituted (50 μL) using 0.1% TFA in 30% ACN (aq). One μL of the above processed samples (n = 12/group) was spotted (N = 8) on a 384/600 anchor chip target plate (Bruker Daltonics, Bremen, Germany). One μL of matrix solution (10 mg/mL α-cyano-4-hydroxy cinnamic acid in 50% acetonitrile, 0.1% TFA) was added on the sample spot and was mixed as described previously [34,35]. An on-target washing of the sample spot was carried out by placing 2.5 μL of cold 1% TFA in water on the dried sample spot, and the liquid was removed after 10 s. Washed spots were dried and analyzed by MALDI-TOF–TOF-MS using a Bruker Daltonics Autoflex III time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a Smart BeamTM laser (355 nm wavelength), a 1 GHz sampling rate digitizer, a pulsed ion extraction source, and a TOF–TOF-MS analyzer. Calibration was done using external protein and peptide calibration standards (m/z range of 1000 to 6000 Da; Bruker). Detection was carried out both in linear and reflectron positive modes. In a typical experiment, a composite spectrum (total of 4000 shots) was obtained by summation of twenty 200-shots of individual spectra. The sampling sites were selected randomly for every sample in order to obtain homogenous sampling. Eight spots/sample were analyzed to enhance overall homogeneity of sampling to obtain the optimal representative mass spectra of the sample (for spot to spot reproducibility among samples please see Supplementary Fig. 1). The data acquisition and processing were carried out using the Flex Control 3.3 and Flex analysis 3.3 software (Bruker Daltonics, Bremen, Germany), respectively. Post-processed mass spectral data (m/z values from the MS scan) were mined for candidate biomarkers [34] by building discriminatory models (ClinPro Tools software version 2.2, Bruker Daltonics, Bremen, Germany), and by statistical analysis. Potential markers were subjected to MS/MS analyses in "Lift" mode for protein identification. Lift conditions used in this study were, ion source 1–6.00 kV; ion source 2–5.30 kV; lens – 3.00 kV; reflectron 1–26.94 kV; reflectron 2–11.48; lift 1–18.97 kV; lift 2 – 3.77 kV. The MS/MS data were queried against SwissProt, NCBInr databases with BioTools software (Bruker Daltonics, Bremen, Germany) using the MASCOT search engine for protein identification. Number of allowed missed cleavages was 1 and the variable modification allowed was methionine oxidation, also decoy sequences were included (FDR < 1).

2.7. Target protein marker analysis

Target markers included in this study were protein markers relevant to inflammatory and endothelial injury pathways which are some of the mechanisms associated with adverse birth outcomes, especially low birth weight outcome, based on previous work as stated above.

2.8. Affinity-based multiplex protein array analyses

Analysis of maternal plasma samples for target markers related to endothelial dysfunction, inflammation and oxidative stress such as cytokines (TNF-α, IFN-γ, IL-2, IL-6, IL-8, IL10, IL12), chemokines (MCP-1, MIP-1β), cellular adhesion molecules (VCAM, ICAM), matrix metalloproteinases (MMP-1, MMP-2, MMP7, MMP-9, MMP-10) and vascular endothelial growth factor (VEGF) were conducted by affinity-based multiplex protein array analysis (Biorad, Millipore) based on the procedure reported by Surronen et al., 2010 [36].

2.9. Circulating endothelin isoforms

This procedure was conducted as described before [33]. Briefly, aliquots of 3rd trimester maternal plasma samples (250 μL) were treated with 3,4-dichloroisocoumarin solution in isopropanol to prevent conversion of big ET-1 to ET-1 during sample processing. These samples were then deproteinized with acidified acetone, followed by clean-up using molecular weight cut-off filters (30 kDa). Clarified samples were dried under a N_2 flow, and were reconstituted in the mobile phase A (composition is given below), and were analyzed by a reversed phase HPLC-Fluorescence system. Initial separation of endothelin isoforms (Big ET-1, ET-1, ET2 and ET-3) were carried out on a LC-318 column (25 cm length, 4.6 mm id, 5 μm particle size; Supelco, Oakville, ON) by gradient elution using water-acetonitrile mobile phase (A-30% acetonitrile (aq); B-90% acetonitrile (aq)) with 0.19% of TFA used as the ion-pair reagent. Analytes were measured by fluorescence detection at excitation and emission wavelengths of 240 nm and 380 nm, respectively.

2.10. Statistics and bioinformatic analyses

Post-processed 3rd trimester maternal plasma global proteomic (m/z) data were mined for significant peptide changes and discriminatory model building (ClinPro Tools version 2.0, Bruker) and candidate biomarker identification [34]. Target protein biomarker levels in the corresponding maternal plasma samples were tested by one-way ANOVA using infant birth weight (low vs normal) as a factor (SigmaStat v3.5, SPSS Inc., Chicago, IL). Differences between the maternal plasmatic proteomic changes for the target biomarkers were determined by one-way analysis of variance (ANOVA) using infant birth weight (low vs normal) as a factor (SigmaStat v3.5, SPSS Inc., Chicago, IL). In order to visualize the differential pattern of responses a heat map with hierarchical clustering was constructed using the Heat map software (Los Alamos National Laboratory, Los Alamos, NM, USA; http://www.hiv.lanl.gov/content/sequence/HEATMAP/heatmap_mainpage.html). Protein interaction networks and biofunctions were identified using Ingenuity Pathway Analysis (IPA) (Ingenuity Systems, www.ingenuity.com) based on target and global protein marker ($p < 0.05$) changes.

3. Results and discussion

Adverse pregnancy outcomes such as fetal growth restriction, preterm birth, low birth weight, birth defects, perinatal and infant death have been associated to a number of maternal and prenatal factors including environmental contaminant exposures [4–20,37–39]. Moreover, maternal physiological changes have been shown to influence birth outcomes [13,17]. In this study, a small subset of maternal plasma samples from the MIREC cohort were analyzed by global shotgun screening and targeted analyses for proteomic changes to determine the efficiency of these biomarker approaches in discriminating maternal physiological conditions relevant to adverse pregnancy outcomes. Here, we have defined the low infant birth weight as the low 10th percentile of birth weight in this mother–infant cohort, <2.7 kg to gain power in the low infant birth weight group (Fig. 1). Also, in this preliminary work, we have attempted to use equal number of cases (mothers with infants of low birth weights) and controls (mothers with infants of normal birth weights). Gestational age and maternal blood pressure profiles associated with these two groups are illustrated in Fig. 1. These results suggest comparably low gestational age ($p < 0.001$) in the low birth weight group as compared to the controls. Also, both mean systolic and diastolic blood pressure levels in mothers are relatively higher in the low infant birth weight group compared to the control group, but did not reach statistical significance in this small sample size.

For the shotgun proteomic screening analysis of plasma, we chose to conduct a very simple plasma fractionation strategy with minimal sample handling steps, and used adequate stabilizers to prevent any post-sample collection changes to avoid any experimental artifacts and potential sample losses due to any recovery issues. Retaining the integrity of plasma proteins was essential in order to gain a clear preliminary understanding of maternal physiological changes that can be specific to adverse birth outcomes. Our results on global high content proteomic data from the MS scans of maternal plasma suggested that there were up or down regulation of various proteins in mothers with low infant birth weights compared to the ones with normal birth weight babies. The m/z data was post-processed using ClinProTools software from Bruker Daltonics with subsequent discriminatory model building as mentioned above. Post-processing of raw mass spectral data included normalization with respect to total ion counts (TIC). Also, significant changes were verified by additional independent statistical analysis (one-way ANOVA) for heat map construction. Statistically significant ($p < 0.05$) proteomic changes summarized as tryptic peptide heat maps with hierarchical clustering (Fig. 2) indicated that maternal plasma global proteomic information at the third trimester can discriminate between those mothers who subsequently gave birth to infants with low birth weights, by comparison to mothers who gave birth to normal birth weight infants. Our results suggest that misclassification into low or normal birth weight groups can be identified by comparing these proteomic patterns (Fig. 2), for example, mothers with the infant birth weights of 2672 g and 3750 g appear to be misclassified based on the infant birth weight concept. However, their proteomic patterns suggest that the classification should be reversed. This reversal of classification can be due to various factors. It is plausible that if the mother and the father had relatively small body mass, the infant can be of lower birth weight at term. Similarly, larger parents may have infants with larger birth weight for gestational age. Such discrepancies can be verified by

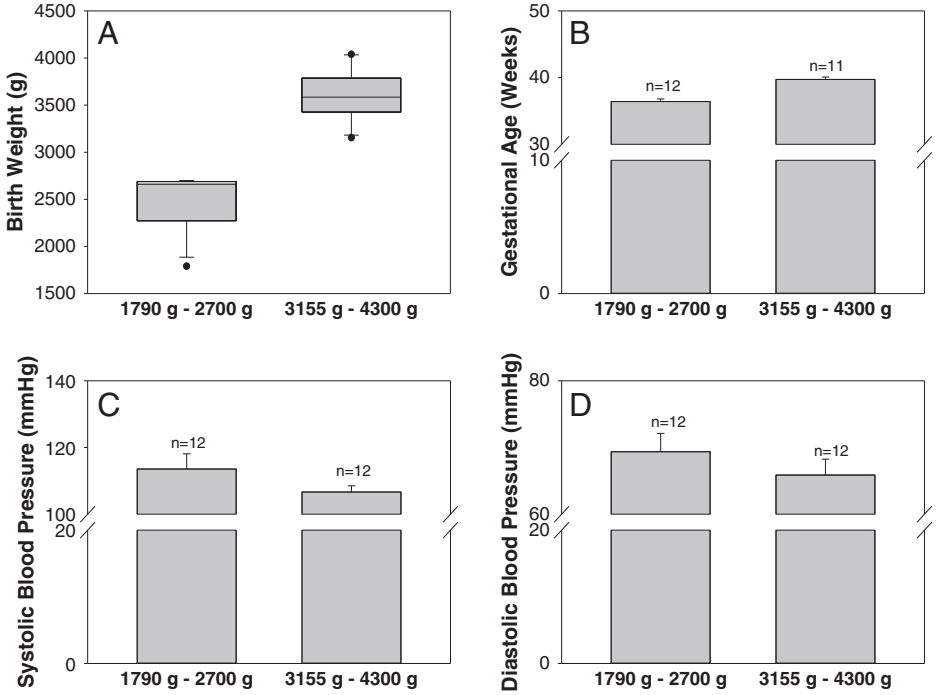


Fig. 1 – Profiles of A. birth weight, B. gestational age and maternal C. systolic and D. diastolic blood pressure levels for the two (low vs normal) infant birth weight groups. Results are expressed as mean ± standard error.

assessment of maternal physiological indices, questionnaires, target marker profiles and other infant parameters. Future analyses of the MIREC data sets will incorporate maternal age, ethnicity and other parameters to determine the causes of similar discrepancies.

A number of candidate tryptic peptide markers (e.g., obtained by discriminatory model building using ClinPro Tools software from Bruker Daltonics, as well as by one-way ANOVA analysis) were subjected to MS/MS analysis, and the fragmentation spectra were matched against SwissProt or/and

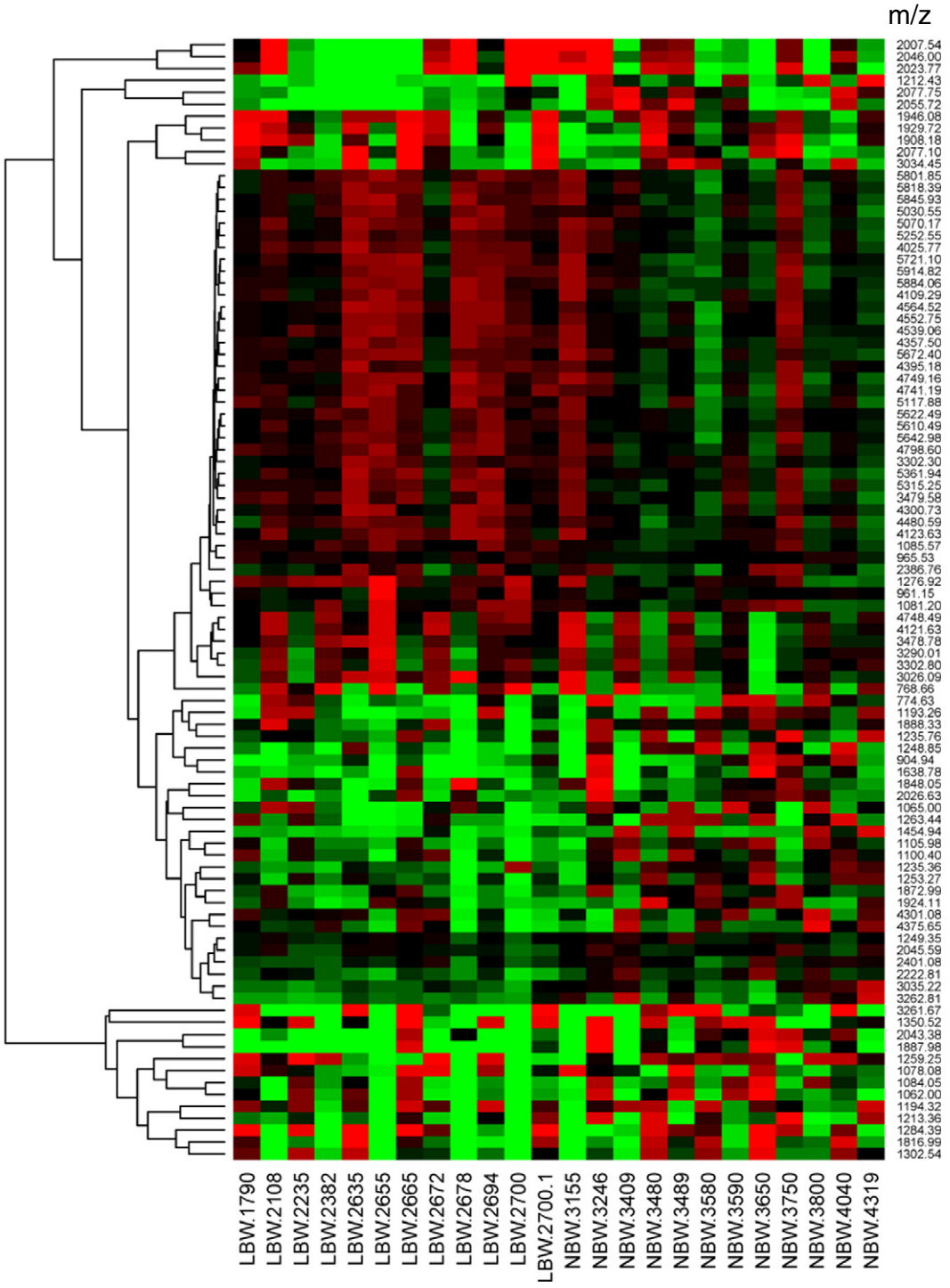


Fig. 2 – Heat map of plasma peptide (m/z) expressions revealed significant ($p < 0.05$) differences between mothers of low birth weight (LBW) and normal birth weight (NBW) infants. Hierarchical clustering of the data reveal clusters of candidate peptides differentially expressed between the two groups. Green indicates down regulation and red indicates up regulation. A yellow-red version of the heatmap is available in supplementary material. Note: (If the yellow–red figure is considered then, yellow indicates down regulation and red indicates up regulation).

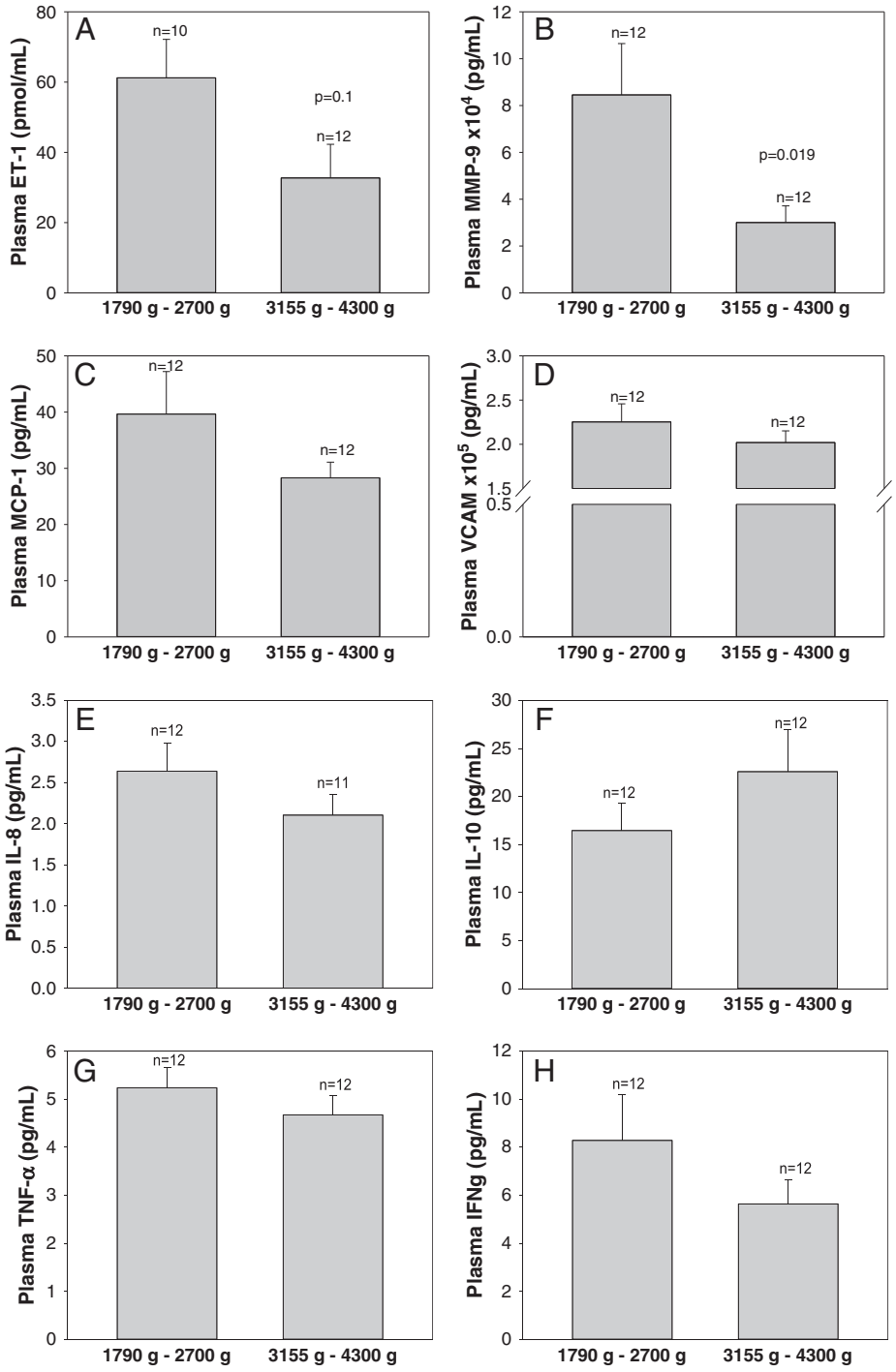


Fig. 3 – Antibody-based multiplex protein array analysis results for the 3rd trimester maternal plasma samples. A. ET-1 B. MMP-9C. MCP-1 D. VCAM E. IL-8F. IL-10G. TNF-α H. IFN-gamma. Results are expressed as mean ± standard error.

NCBInR databases, using MASCOT search engine, for protein identification. Although statistically strongest assignments with the highest ions score, low expect value and unique peptide were used as criteria for potential protein identification, the best matches with low scores were retained if they were unique peptides and were biologically relevant or significant (Supplementary material, Table S1). In this work, our primary aim was to test the discriminatory capability of the global proteomic strategy. In this proof-of-principle effort, the most significant candidate protein markers identified as mentioned above were used as secondary addition to the primary data on target protein markers to enhance the confidence in the networks selected by the IPA analysis.

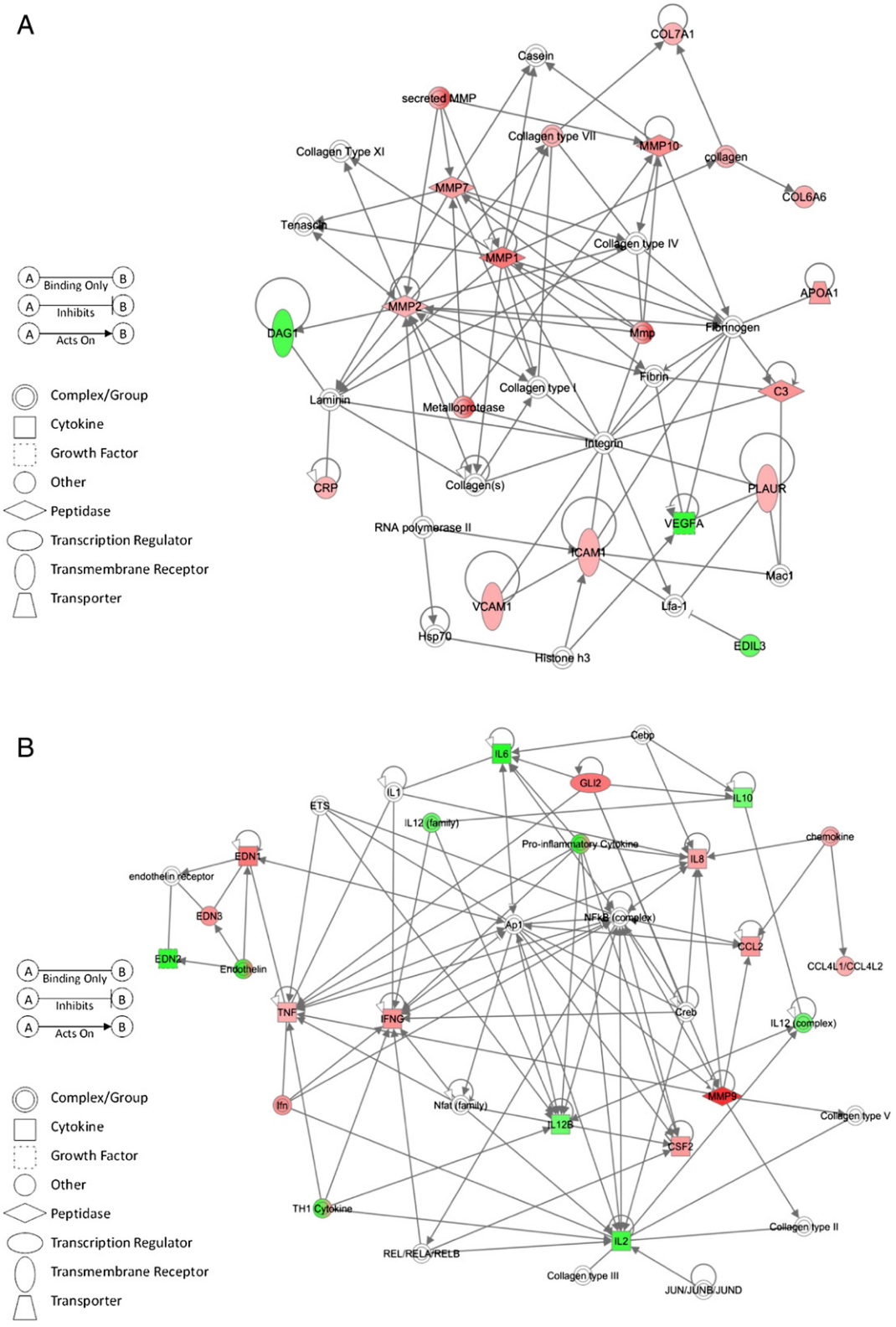


Fig. 4 – Results of IPA analysis performed based on fold changes in maternal plasma levels (low vs normal infant birth weight groups) of both target and global protein biomarkers (A) NETWORK 1: Ophthalmic Disease, Cardiac Stenosis, Cardiovascular Disease (Score 30). (B) NETWORK 2: Cellular Movement, Hematological System Development and Function, Immune Cell Trafficking (Score 29). Green indicates down regulation and red indicates up regulation.

Of the target markers analyzed, a subset of marker profiles are illustrated in Fig. 3. The proinflammatory cytokines IL-8, TNF-alpha, and IFN-gamma were all increased, while the anti-inflammatory cytokine IL-10 was decreased, in plasma of mothers associated with low birth weight infants suggesting a proinflammatory state in these mothers. Similarly, plasmatic ET-1, matrix metalloproteinase (e.g. MMP-9), MCP-1 and VCAM implicated in cardiovascular diseases were elevated [40]. We have previously shown [33,41,42] that in animals and humans exposed to air pollutants there is an increase in circulating endothelin isoforms (ET-1). ET-1 is a potent vasoconstrictor peptide and is formed as a result of the cleavage of its precursor peptide big ET-1 that is comparably less potent by the action of endothelin converting enzyme (ECE).[33] ET-1 is implicated in gestational hypertension and gestational diabetes [43]. Similarly, matrix metalloproteinases are evolving as new set of markers of endothelial injury and are implicated in downstream signaling of vasoregulatory events such as increase in circulating ET-1 levels [44,45].

IPA analysis with target and global protein biomarker information generated several protein interaction networks. Two of these networks with the highest scores are illustrated in Fig. 4A–B. The networks identified here are associated with ophthalmic, cardiovascular, dermatological and neurological conditions, cellular movement, immune cell trafficking, hematological system development, small molecule biochemistry, lipid metabolism and molecular transport. It is noteworthy to see that urokinase plasminogen activator receptor (PLAUR) in network 1 (cardiovascular diseases; Fig. 4A) is up-regulated in mothers with low birth weight infants in our preliminary work. It has been shown by others that elevated levels of plasma urokinase plasminogen activator receptor in mothers infected with malaria (pro-inflammatory condition) is predictive of low infant birth weight.[46] Also, our results exhibit that matrix metalloproteinases (MMPs) are up-regulated (network 1, cardiovascular diseases, Fig. 4A; network 2, immune cell trafficking, Fig. 4B) in mothers with low birth weight infants. This is in line with the report by Sundrani et al., 2011, where MMPs are implicated in the pathophysiology of adverse pregnancy outcomes such as preterm labor that may result in low birth weight infants.[47] The networks that we have observed in this study also trigger detailed analysis of their components for which we do not have data currently but can be achieved through future affinity-based analyses. Furthermore, when related to information on biological functions and diseases, IPA analyses yielded profiles of molecular and cellular functions, diseases/disorders and physiological system development and functions with associated significance values. These preliminary results imply cell signaling, cellular movement, molecular transport, organismal injury and abnormalities, inflammatory, respiratory and cardiovascular effects-related changes in mothers with low birth weight infants compared to the ones with normal birth weight babies. Nevertheless, these findings have to be further investigated through future work on much larger set of samples.

Shot-gun proteomic methodologies face challenges in terms of protein recoveries and experimental artifacts due to intensive sample processing steps, issues of internal standards, necessity of high-end analytical platforms and bottle neck in data handling due to explosion in data generation [31]. Nevertheless, our results demonstrate that global shotgun proteomic approach is a promising tool in understanding negative pregnancy outcomes. We intend to apply this approach to advance our knowledge of environmental contaminant exposure-mediated toxicity mechanisms that can potentially lead to adverse pregnancy outcomes, and impacts of nutritional intervention on these mechanistic pathways, through future work. Increased sample size along with LC-based separation of plasma followed by MS/MS analyses and enhanced protein identification/validation processes with emerging analytical and bioinformatics tools can advance the application of such global proteomic analyses to similar complex investigations.

4. Conclusion

High-throughput shotgun screening of global plasma proteomic changes is useful in discriminating mothers with different physiologies that are related to low birth weight (<2700 g), a potentially adverse pregnancy outcome. Considerable metabolic control is executed at the metabolite as well as the protein levels including post-translational modifications, constituting phenotypic plasticity. Our results indicate that a mass spectrometry-based proteomic approach can capture molecular phenotypes and can be of utility for the identification of prognostic biomarkers of adverse pregnancy outcomes. Combined use of the information from target biomarkers and high-content global proteomics should provide valuable insight into adverse outcome pathways.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jprot.2013.12.003.

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REFERENCES

[1] Stillerman KP, Mattison DR, Giudice LC, Woodruff TJ. Environmental exposures and adverse pregnancy outcomes: a review of the science. Reprod Sci 2008;15(7):631–50.

[2] Perera FP, Tang D, Rauh V, Tu YH, Tsai WY, Becker M, et al. Relationship between polycyclic aromatic hydrocarbon-DNA adducts, environmental tobacco smoke, and child development in the World Trade Center cohort. Environ Health Perspect 2007;115(10):1497–502.

[3] Woodruff TJ, Parker JD, Darrow LA, Slama R, Bell ML, Choi H, et al. Methodological issues in studies of air pollution and reproductive health. Environ Res 2009;109(3):311–20.

[4] Wigle DT, Arbuckle TE, Turner MC, Berube A, Yang Q, Liu S, et al. Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. J Toxicol Environ Health B Crit Rev 2008;11(5–6):373–517.

[5] Mathews F, Yudkin P, Neil A. Influence of maternal nutrition on outcome of pregnancy: prospective cohort study. BMJ 1999;319(7206):339–43.

[6] Jolly M, Sebire N, Harris J, Robinson S, Regan L. The risks associated with pregnancy in women aged 35 years or older. Hum Reprod 2000;15(11):2433–7.

[7] Gelson E, Johnson M. Effect of maternal heart disease on pregnancy outcomes. Expert Rev Obstet Gynecol 2010;5(5):605–17.

[8] Alsuwaida A, Mousa D, Al-Harbi A, Alghonaim M, Ghareeb S, Alrukhaimi MN. Impact of early chronic kidney disease on maternal and fetal outcomes of pregnancy. J Matern Fetal Neonatal Med 2011;24(12):1432–6.

[9] Abu-Saad K, Fraser D. Maternal nutrition and birth outcomes. Epidemiol Rev 2010;32(1):5–25.

[10] Godfrey K, Robinson S, Barker DJ, Osmond C, Cox V. Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. BMJ 1996;312(7028):410–4.

[11] King JC. The risk of maternal nutritional depletion and poor outcomes increases in early or closely spaced pregnancies. J Nutr 2003;133(5 Suppl 2):1732S–6S.

[12] Campbell DM, Hall MH, Barker DJ, Cross J, Shiell AW, Godfrey KM. Diet in pregnancy and the offspring's blood pressure 40 years later. Br J Obstet Gynaecol 1996;103(3):273–80.

[13] Kenny LC, Lavender T, McNamee R, O'Neill SM, Mills T, Khashan AS. Advanced maternal age and adverse pregnancy outcome: evidence from a large contemporary cohort. PLoS One 2013;8(2):e56583.

[14] Chantrapanichkul P, Chawanpaiboon S. Adverse pregnancy outcomes in cases involving extremely young maternal age. Int J Gynaecol Obstet 2013;120(2):160–4.

[15] Howe LD, Matijasevich A, Tilling K, Brion MJ, Leary SD, Smith GD, et al. Maternal smoking during pregnancy and offspring trajectories of height and adiposity: comparing maternal and paternal associations. Int J Epidemiol 2012;41(3):722–32.

[16] Hackshaw A, Rodeck C, Boniface S. Maternal smoking in pregnancy and birth defects: a systematic review based on 173 687 malformed cases and 11.7 million controls. Hum Reprod Update 2011;17(5):589–604.

[17] Stangl V, Schad J, Gossing G, Borges A, Baumann G, Stangl K. Maternal heart disease and pregnancy outcome: a single-centre experience. Eur J Heart Fail 2008;10(9):855–60.

[18] Sugamata M, Ihara T, Sugamata M, Takeda K. Maternal exposure to diesel exhaust leads to pathological similarity to autism in newborns. J Health Sci 2006;52(4):486–8.

[19] Lin YY, Hwang YH, Chen PC, Chen BY, Wen HJ, Liu JH, et al. Contribution of gestational exposure to ambient traffic air pollutants to fetal cord blood manganese. Environ Res 2012;112:1–7.

[20] Maroziene L, Grazuleviciene R. Maternal exposure to low-level air pollution and pregnancy outcomes: a population-based study. Environ Health 2002;1(1):6.

[21] Arslan M, Yazici G, Erdem A, Erdem M, Arslan EO, Himmetoglu O. Endothelin 1 and leptin in the pathophysiology of intrauterine growth restriction. Int J Gynaecol Obstet 2004;84(2):120–6.

[22] Triche EW, Hossain N. Environmental factors implicated in the causation of adverse pregnancy outcome. Semin Perinatol 2007;31(4):240–2.

[23] Tabacova S, Baird DD, Balabaeva L. Exposure to oxidized nitrogen: lipid peroxidation and neonatal health risk. Arch Environ Health 1998;53(3):214–21.

[24] Gitto E, Reiter RJ, Karbownik M, Tan DX, Gitto P, Barberi S, et al. Causes of oxidative stress in the pre- and perinatal period. Biol Neonate 2002;81(3):146–57.

[25] Cindrova-Davies T. Gabor Than Award Lecture 2008: pre-eclampsia—from placental oxidative stress to maternal endothelial dysfunction. Placenta 2009;30(Suppl. A):S55–65.

[26] Bell ML, Ebisu K, Belanger K. Ambient air pollution and low birth weight in Connecticut and Massachusetts. Environ Health Perspect 2007;115(7):1118–24.

[27] Burton GJ, Jauniaux E. Placental oxidative stress: from miscarriage to preeclampsia. J Soc Gynecol Investig 2004;11(6):342–52.

[28] Madazli R, Atis A, Uzun H, Aksu F. Mid-trimester amniotic fluid angiogenin, lactate dehydrogenase and fibronectin in the prediction of preterm delivery. Eur J Obstet Gynecol Reprod Biol 2003;106(2):160–4.

[29] Gilmore JM, Washburn MP. Advances in shotgun proteomics and the analysis of membrane proteomes. J Proteomics 2010;73(11):2078–91.

[30] Fabietti A, Gaspari M, Krishnan S, Quirino A, Liberto MC, Cuda G, et al. Shotgun proteomic analysis of two Bartonella quintana strains. Proteomics 2013;13(8):1375–8.

[31] Wu CC, MacCoss MJ. Shotgun proteomics: tools for the analysis of complex biological systems. Curr Opin Mol Ther 2002;4(3):242–50.

[32] Arbuckle TE, Fraser WD, Fisher M, Davis K, Liang CL, Lupien N, et al. Cohort profile: the maternal-infant research on environmental chemicals research platform. Paediatr Perinat Epidemiol 2013;27(4):415–25.

[33] Kumarathasan P, Goegan P, Vincent R. An automated high-performance liquid chromatography fluorescence method for the analyses of endothelins in plasma samples. Anal Biochem 2001;299(1):37–44.

[34] Kumarathasan P, Das D, Salam MA, Mohottalage S, DeSilva N, Simard B, et al. Mass spectrometry-based proteomic assessment of the in vitro toxicity of carbon nanotubes. Curr Top Biochem Res 2012;14(1):15–27.

[35] Kumarathasan P, Mohottalage S, Goegan P, Vincent R. An optimized protein in-gel digest method for reliable proteome characterization by MALDI-TOF-MS analysis. Anal Biochem 2005;346(1):85–9.

[36] Suuronen EJ, Hazra S, Zhang P, Vincent R, Kumarathasan P, Zhang Y, et al. Impairment of human cell-based vasculogenesis in rats by hypercholesterolemia-induced endothelial dysfunction and rescue with L-arginine supplementation. J Thorac Cardiovasc Surg 2010;139(1):209–16.

[37] Shah NR, Bracken MB. A systematic review and meta-analysis of prospective studies on the association between maternal cigarette smoking and preterm delivery. Am J Obstet Gynecol 2000;182(2):465–72.

[38] Grandjean P, Bellinger D, Bergman A, Cordier S, Davey-Smith G, Eskenazi B, et al. The faroes statement: human health effects of developmental exposure to chemicals in our environment. Basic Clin Pharmacol Toxicol 2008;102(2):73–5.

[39] Bellinger DC. A strategy for comparing the contributions of environmental chemicals and other risk factors to neurodevelopment of children. Environ Health Perspect 2012;120(4):501–7.
[40] Boyle JJ. Macrophage activation in atherosclerosis: pathogenesis and pharmacology of plaque rupture. Curr Vasc Pharmacol 2005;3(1):63–8.
[41] Vincent R, Kumarathasan P, Goegan P, Bjarnason SG, Guenette J, Berube D, et al. Inhalation toxicology of urban ambient particulate matter: acute cardiovascular effects in rats. Res Rep Health Eff Inst 2001;104:5–54.
[42] Calderon-Garciduenas L, Vincent R, Mora-Tiscareno A, Franco-Lira M, Henriquez-Roldan C, Barragan-Mejia G, et al. Elevated plasma endothelin-1 and pulmonary arterial pressure in children exposed to air pollution. Environ Health Perspect 2007;115(8):1248–53.
[43] Lygnos MC, Pappa KI, Papadaki HA, Relakis C, Koumantakis E, Anagnou NP, et al. Changes in maternal plasma levels of VEGF, bFGF, TGF-beta1, ET-1 and sKL during uncomplicated pregnancy, hypertensive pregnancy and gestational diabetes. In Vivo 2006;20(1):157–63.
[44] Xu J, Zou MH. Molecular insights and therapeutic targets for diabetic endothelial dysfunction. Circulation 2009;120(13):1266–86.
[45] Schafers M, Schober O, Hermann S. Matrix-metalloproteinases as imaging targets for inflammatory activity in atherosclerotic plaques. J Nucl Med 2010;51(5):663–6.
[46] Ostrovsky SR, Shulman CE, Peshu N, Staalsoe T, Hoyer-Hansen G, Pedersen BK, et al. Elevated plasma urokinase receptor predicts low birth weight in maternal malaria. Parasite Immunol 2007;29:37–46.
[47] Sundrani DP, Chavan Gautam PM, Mehendale SS, Joshi SR. Altered metabolism of maternal micronutrients and omega 3 fatty acids epigenetically regulate matrix metalloproteinases in preterm pregnancy: a novel hypothesis. Med Hypotheses 2011;77:878–83.