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Alterations in maternal plasma protein profiles in pregnant women with asthma

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Role of authors

Vanessa Murphy was involved in study design, protocol development, conducting SELDI experiments, data analysis and manuscript preparation.

Renee Johnson was involved in protocol development, conducting SELDI experiments, data review and manuscript review.

Yung-Chih Wang was involved in protocol development, data review and manuscript review.

Karen Akinsanya was involved in protocol development, data review and manuscript review.

Peter Gibson was involved in subject characterization, data review and manuscript review.

Roger Smith was involved in study design, protocol development, data review and manuscript review.

Vicki Clifton was involved in study design, protocol development, data review and manuscript review.

Abstract

Objective and Background:

The course of asthma may be altered during pregnancy with at least one third of women experiencing a worsening of asthma and 20% having an exacerbation during pregnancy. We used the novel proteomic technique, surface enhanced laser desorption ionization-time of flight mass spectrometry (SELDI-TOF MS) to determine if the presence of asthma during pregnancy was associated with alterations in plasma proteins.

Methodology:

Plasma collected from healthy (n=23) and asthmatic (n=27) pregnant women at 18 and 30 weeks gestation was applied to strong anion exchange (SAX2), weak cation exchange (WCX2) and immobilized metal affinity capture (IMAC-Cu²⁺) chips. Mass analysis was conducted using Ciphergen Protein Biology System IIc and significant differences in individual peak intensities between groups determined.

Results:

At 18 weeks gestation, 91 peaks were significantly different between pregnant women with and without asthma, representing 28% of the total peaks identified. At 30 weeks gestation, 51 peaks were significantly different. There were 2 peaks which were significantly different between groups at both 18 and 30 weeks gestation and expressed at a similar level at both times points. One was increased in asthmatics (molecular weight = 6444); the other decreased in asthmatics compared to non-asthmatic women (molecular weight = 1846).

Conclusions:

Our study demonstrated that there are differences in protein patterns between pregnant women with and without asthma. Other techniques would be required to define the molecular species and classify pathophysiological significance. SELDI-TOF MS has potential as a tool to monitor disease progression in situations such as pregnancy.

Key Words: SELDI, proteomics, asthma, pregnancy, plasma

Short Title: Plasma proteins in asthmatic pregnancies

Introduction

Asthma is the most common chronic illness to complicate pregnancy, affecting at least 12% of pregnant women in Australia [1]. Asthma during pregnancy can contribute to significant morbidity for both mother and fetus, in the form of low birth weight [2-5], preterm delivery [3-6] and pre-eclampsia [4, 6]. At least one third of women experience worsening asthma during pregnancy [7] and 20% have an exacerbation requiring medical intervention during pregnancy [8]. Currently, our understanding of the mechanisms which contribute to pregnancy-induced changes in asthma, or to poor pregnancy outcomes in women with asthma are not well understood.

Proteomics is a growing area for studying protein products expressed by the genome of an organism, tissue or cell type [9], which may change in response to developmental stage, environment or disease [9]. Proteomic studies in asthma research are limited, however a recent review indicated that while proteomic discovery approaches are less well developed than genomic approaches, they have the potential to generate novel biomarkers of asthma susceptibility or asthma severity in the future [10].

Surface enhanced laser desorption ionization-time of flight mass spectrometry (SELDI-TOF MS) is a novel proteomic technique [11] which combines on-chip retentate chromatography with mass spectrometry [12]. Proteins in crude biological samples differentially bind to chip surfaces based upon their chemical and biochemical properties such as hydrophobicity or charge [13]. The specific interactions between the protein and chip surface are based upon the amino acid sequence and structure of the protein, and the detergent, salt concentration and pH of the binding buffer [14]. Proteins retained on the chip become embedded in an energy absorbing molecule (EAM) or matrix, which is

ionized by a laser [12]. The gaseous ions move through a vacuum towards a detector and the time taken to reach the detector is used to derive the mass to charge (m/z) ratio [12]. SELDI-TOF MS is useful for detecting low mass proteins and peptides (<20 kDa), but can also detect large proteins up to 150 to 200 kDa [13].

In this study, we used SELDI-TOF MS to profile proteins present in maternal plasma collected at 18 and 30 weeks gestation from pregnant women with and without asthma. The aim of this initial analysis was to examine the extent of plasma protein profile alterations in pregnant women with asthma, compared to healthy women. We also wished to determine whether SELDI-TOF MS was a useful screening method for generating novel biomarker candidates, which could be further characterized and used for diagnostic monitoring or testing in the future.

Methods

Approval for the study was provided by the Hunter Area Health Service and University of Newcastle Human Research Ethics Committees. Pregnant women with (n=27) and without (n=23) a doctor diagnosis of asthma were recruited at the John Hunter Hospital antenatal clinic as previously described [15-17]. The patient's clinical history of asthma and spirometry results were reviewed by the Respiratory physician (PGG).

The majority of subjects were classified during the study as having either mild, moderate or severe asthma based on the integrated severity score, described in the Australian asthma management guidelines which are similar to the guidelines from the National Heart, Lungs and Blood Institute [18, 19]. Women were assigned a severity rating of mild, moderate or severe according to symptoms, asthma history and other features including forced expiratory volume at one second (FEV₁) and peak expiratory flow (PEF). Women were assigned to the most severe category which applied for any one of the following criteria at their first study visit. Women with mild asthma were characterised by an FEV₁ >80% predicted, <25% variability in PEF, no night time or morning symptoms, infrequent β_2 -agonist use, daytime symptoms <4 times per week and no severe attacks in the previous year. Women with moderate asthma were characterised by an FEV₁ of 60-80% predicted, <25% PEF variability, night time symptoms up to once per week, β_2 -agonist use and daytime symptoms on most days. Women with severe asthma were characterised by an FEV₁ <60% predicted, >25% variability in PEF, frequent night time symptoms, morning and daytime symptoms every day, β_2 -agonist use 3-4 times per day and limited physical activity. Most patients (68%) were classified as having mild asthma (Table 1).

Maternal plasma was collected into lithium heparin tubes at approximately 18 and 30 weeks gestation and stored at -20°C until use. Plasma samples were thawed once on ice, centrifuged, aliquoted into 100 µl portions and stored at -80°C prior to use in SELDI experiments. None of the non-asthmatic subjects had more than one blood sample taken, while 12 of the asthmatic subjects had a sample taken at both 18 and 30 weeks.

Proteomic analysis (SELDI-TOF MS) was conducted with the Protein Biology System IIc and ProteinChip® software version 3.1.1, biomarker edition (Ciphergen, Freemont, CA). Each day, low and high mass calibrations were performed using all-in-1 peptide molecular weight and all-in-1 protein standards (Ciphergen, Freemont, CA) respectively. At least 4 of the 5 standard peptides or proteins were used to derive the calibration equation. The low mass molecular weight standard consisted of Arg-vasopressin (1084.2 Da), somatostatin (1637.9 Da), bovine insulin β-chain (3495.9 Da), human insulin (5807.7 Da) and hirudin (7033.6 Da). The high mass molecular weight standard consisted of equine cytochrome C (12360.2 Da), equine cardiac myoglobin (16951.5 Da), rabbit glyceraldehyde-3-phosphate dehydrogenase (35688 Da), bovine serum albumin (66433 Da) and Escherichia coli β-galactosidase (116351 Da).

Plasma samples were thawed on ice and centrifuged at 8000 g for 10 minutes at 4°C prior to addition to strong anion exchange (SAX 2), weak cation exchange (WCX 2) and immobilized metal affinity capture (IMAC-Cu²⁺) ProteinChip® surfaces. Each day only one chip type was used and 8 spot chips were processed in a 12 array bioprocessor (Ciphergen, Freemont, CA).

ProteinChip[®] arrays were activated, neutralized and equilibrated with binding buffer prior to sample addition. IMAC chips were pre-activated with 50 µl CuSO₄ per spot and WCX chips activated with 100 µl 10 mM HCl per spot, by shaking for 15 minutes. SAX chips were not activated. Following a distilled water rinse, IMAC chips were neutralized with 100 µl 50 mM sodium acetate (pH 4), WCX chips with 100 µl ammonium acetate (pH 6) and 100 µl 50 mM Tris (pH 9) was added to SAX chips and shaken for 5 minutes. After rinsing, the chips were equilibrated with 150 µl of low stringency binding buffer (0.1 M ammonium acetate + 0.1% triton X-100, pH 4 for WCX, phosphate buffered saline + 0.5 M NaCl + 0.1 % triton X-100, pH 7 for IMAC and 0.05 M Tris + 0.1% triton X-100, pH 9 for SAX). Following two washes with binding buffer, 100 µl of fresh buffer and 2.5 µl of plasma was added to each spot and incubated for 30 minutes at room temperature on a shaking platform. Sample was removed and wells rinsed three times with the appropriate binding buffer (200 µl per well, 5 minutes each wash). After two further washes with distilled water, individual arrays were removed from the bioprocessor and air-dried. 3,5-dimethoxy-4-hydroxycinnamic acid (SPA) and α -cyano-4-hydroxycinnamic acid (CHCA) were used as energy absorbing molecules (EAM). Saturated solutions of SPA and CHCA were made up daily with 100 µl acetonitrile and 100 µl of 1% trifluoroacetic acid, vortexed for 3 minutes and centrifuged at 8000 g for 3 minutes at room temperature. 0.5 µl of SPA or CHCA was added twice to each spot and allowed to air dry between applications.

When SPA was used as the EAM, mass analysis was performed at a laser intensity of 195 and 220 for low and high mass proteins respectively. When CHCA was used, the laser intensity was set at 170 with only low mass peptides detected. Baseline subtraction was performed on all spectra. Protein profiles to be compared were normalized for total ion

current before proceeding. Regions prior to 1500 Da for low mass spectra, and 10000 Da for high mass spectra, were considered noise. The Biomarker Wizard found new clusters by selecting peaks with a signal/noise ratio >5 on the first pass and >2 on the second pass. The minimum peak threshold was set at 20%, signifying that 20% of all spectra must contain the peak in order for it to be considered a valid cluster. The cluster mass window was set at 0.3% of mass, signifying that all peaks considered to be from the same cluster must be within 0.3% of each other's mass.

Statistical differences in protein peak intensities between groups was determined using the Biomarker Wizard software, which used the parametric two tailed t test or the non-parametric Mann Whitney U test, with $P < 0.05$ considered significant. Maternal subject characteristics of the control and asthma group were compared using the unpaired t test for parametric data and the Mann Whitney test for non-parametric data (GraphPad Instat version 3.05, GraphPad Software, San Diego, CA).

All peaks were visually checked for outliers, possible chemical modifications, consistency between individuals (the same area of the spectrum labeled) and for their relationship to other peaks (for example; doubly charged versions of another peak). Each peak which was significantly different between the groups was assigned a rating of Category A, B or C, which was based on the visual checks and subjectively indicated the quality of each result. Category A peaks were considered highly suitable for follow-up. Category B peaks were suitable for follow-up but may have been related to or a modification of another peak. Category C peaks were of less quality, either due to their shape (too flat or broad) or proximity to a region of noise, or the presence of outliers (not removed) and were considered unsuitable for follow-up. Peaks that were significantly different between

groups, but did not pass the visual checks were excluded. Possible identities of peaks of interest was investigated by searching the Swiss-Prot protein database (<http://us.expasy.org/tools/tadident.html>). The molecular weight (MW) was estimated to be the m/z with 0.5% error. An estimate of the pI of the protein was made, based upon its binding to the chip surfaces. If the peak was found on a WCX chip at pH 4, the pI was assumed to be approximately ≥ 4 . If the peak was found on a SAX chip at pH 9, the pI was assumed to be approximately ≤ 9 . Peaks which bound to IMAC at pH 7 were assumed to have any possible pI. The search was narrowed to include proteins from *homo sapiens* only.

Results

There were no significant differences in maternal age, weight, (unpaired t test, $P>0.05$), height, gravidity, parity or gestational age at delivery between the asthmatic and non-asthmatic groups (Mann Whitney test, $P>0.05$, Table 1).

At 18 weeks gestation, a total of 325 plasma protein peaks were identified. 246 of these were identified on WCX, SAX or IMAC chips using SPA as the EAM, while 79 were identified on WCX, SAX or IMAC chips using CHCA as the EAM (Table 2).

At 18 weeks, there were 91 peaks which were significantly different between plasma of asthmatic ($n=20$) and non-asthmatic women ($n=13$), representing 28% of the total number of protein clusters identified on all chips. Only 12 of these differences were identified on chips using CHCA as the EAM. Of the 91 significant differences between asthmatics and controls at 18 weeks gestation, 28 peaks were considered highly suitable for follow-up (classified as category A peaks, Table 3).

At 30 weeks gestation, a total of 340 plasma protein peaks were identified. 261 of these were identified on WCX, SAX or IMAC chips using SPA as the EAM, while 79 were identified on WCX, SAX or IMAC chips using CHCA as the EAM (Table 2).

At 30 weeks gestation, there were 51 peaks which were significantly different between plasma of asthmatic ($n=19$) and non-asthmatic ($n=10$) women, representing 15% of the total number of protein clusters identified. Using CHCA as the EAM, 19 peak differences were identified, while using SPA identified 32 peak differences. 18 peaks were considered highly suitable for follow-up (Table 4).

We identified only two peaks which were significantly different between the asthma and control groups at both 18 and 30 weeks gestation. One peak was identified using the SAX chip at pH 9, with SPA as the EAM, which was 1.6 times higher in asthmatics compared to controls at both 18 and 30 weeks gestation (Figure 1, t-test, $P=0.005$ at 18 weeks and $P=0.009$ at 30 weeks). The relative peak intensities were similar at both time points, indicating that this is an asthma-associated peptide, which is unaffected by the stage of pregnancy. This peak had an approximate mass to charge ratio (m/z) of 6444 and was classified as category A. Searching the Swiss-Prot database (MW 6444 Da $\pm$ 0.5 %, pI 5 $\pm$ 5) revealed a total of three possible matches. They were pre-T/NK cell associated protein (accession number Q13412), glycophorin E precursor (P15421) and metallothionein-IV (P47944). Further characterization is required to confirm whether this is a novel protein.

One peak was identified using the IMAC chip at pH 7 with CHCA as the EAM which was approximately four to five-fold lower in asthmatics compared to controls at both 18 and 30 weeks gestation (t-test, $P=0.010$ at 18 weeks, $P=0.008$ at 30 weeks, category B). At 30 weeks gestation, the peak appeared to be absent in all but one asthmatic subject. This peak had an approximate m/z of 1846. A search of the Swiss-Prot database (MW 1846 Da $\pm$ 0.5 %, pI 7 $\pm$ 10) revealed one possible match (protachykinin 1 precursor, P20366). However, this peak may represent a novel protein, and further characterization is required to determine the significance of its absence in pregnant asthmatic plasma.

Discussion

In this study, we have successfully used the SELDI-TOF proteomics technique to screen for differences in plasma proteins between asthmatic and non-asthmatic women during pregnancy. This is a novel approach which may lead to the discovery of asthma biomarkers with particular significance during pregnancy. The SELDI-TOF technique is useful as a screening tool, which can identify hundreds of plasma proteins in healthy and diseased subjects. We demonstrated that there are a large number of plasma protein differences between asthmatic and non-asthmatic women during pregnancy. Almost one third of the total number of proteins identified using SELDI-TOF MS were different between the two groups at 18 weeks gestation, while 15% of proteins were different at 30 weeks gestation. The use of SPA as the EAM was clearly better in terms of both the total number of protein peaks identified, and the number of differences between asthmatic and control plasma detected.

We found that only two plasma proteins were consistently different between asthmatics and non-asthmatics during pregnancy. These proteins may be asthma-associated proteins, since their level of expression remained constant across gestation. One peak (m/z 6444) was significantly increased in asthmatics at both stages of pregnancy. The 3 peptides with a possible match to this peak are likely to have roles in asthma, but have not previously been suggested as having diagnostic significance. However, this peak could also represent a novel protein, whose role in asthma is yet to be determined. Such biomarkers of asthma which are not altered by pregnancy may have clinical diagnostic potential and may also be targets for future drug therapies for asthma.

Serum or plasma proteins may be differentially expressed in response to physiological changes such as infection or inflammation [20]. Consequently, the plasma protein

differences observed between women with and without asthma, may have physiological relevance during pregnancy. However, much more biochemical analysis is needed in order to positively identify the peaks of interest and determine their function and significance in this context. In particular, post translational modification or proteolytic cleavage could also result in peptide fragments with similar molecular weights to smaller proteins. It is important to follow this study with further identification by 1-D polyacrylamide gel electrophoresis (PAGE), tryptic digest and MS-MS.

The SELDI-TOF proteomics technique has some advantages over other methods, despite the fact that proteins cannot be positively identified at this stage. SELDI-TOF is a broad screening tool, with the potential to discover novel proteins and novel disease mechanisms. Unlike genomic approaches such as microarray, this proteomics approach is unbiased, in that it does not search only for differences in known proteins. Using a combination of ProteinChip® arrays which capture proteins with different chemical properties, as used in this study, a broad profile of hundreds of proteins can be obtained. Peaks with molecular weights <5 kDa can be characterized, which is not possible using other methods such as 2-D gel electrophoresis [13, 14, 21, 22]. Smaller amounts of sample are required, and less sample processing is needed. In our study, 2.5 µl of plasma was directly bound to each chip, and three different chip surfaces were used with two energy absorbing molecules, providing a simple method for generating a large number of peak differences between groups.

There have been concerns about the reproducibility of SELDI serum profiles [23]. Baggerly *et al.* found that inconsistencies in baseline subtraction and differences in handling or processing between experiments and a lack of regular calibration contributed

to marked variation in results impacting on the conclusions which could be drawn from these studies [23]. They noted that many peaks assumed to be significantly different between healthy and diseased groups, were of a very low molecular weight, and therefore found in the matrix noise region [23]. Further validation and replication of our results are necessary with larger numbers of samples before continuing to pursue the candidate biomarkers we describe here. However, we consider that the results we have obtained in this study are of good quality as systematic differences in sample processing were avoided. For example, we conducted all our experiments in single runs for each chip type, with a single batch of chips and only two operators (VEM, RFJ) were involved in conducting experiments. Daily calibration and baseline subtraction to all spectra were performed. Candidate peaks were all above the noise region, as normalization (>1500 Da for low mass spectra and >10000 for high mass spectra) was performed on all profiles before assessing differences between groups.

SELDI-TOF MS has many applications in both basic research and clinical proteomics. This technology has the potential to identify novel diagnostic markers, monitor disease progression [24] or discover new therapeutic targets [25]. The SELDI-TOF technique has been used in biomarker discovery for diseases such as breast [26] and lung cancer [27], Alzheimer's disease [28] and kidney disease [20]. One problem that has been identified is patient-to-patient variability within disease groups [20]. Some studies have reported results with only 4 patients per group [20], while others have studied profiles in hundreds of patients in order to improve the sensitivity and specificity of the technique as a prediction tool [29]. In our study, we examined profiles of approximately 10 healthy controls and 20 asthmatic subjects. Clearly, future studies should be conducted with a much larger group of

subjects and ideally the asthmatic group should be as homogeneous as possible, comprising women with similar asthma severity and treatment.

It is known that in at least one third of pregnant women, asthma will worsen during gestation [7] and there is currently no way of predicting these changes. A substantial number of women will not follow the same course of asthma, even from one pregnancy to the next [7]. It is possible that a proteomic approach could be used to monitor pregnant patients with asthma in order to detect or predict a worsening condition. Using this approach, it would not be necessary to know the identities of specific proteins of interest, as changes in protein profiles or patterns could be used to detect worsening disease. Proteomics therefore has the potential to lead to improved asthma management strategies for pregnant women, which would ultimately improve the health outcome for both mother and baby.

In conclusion, this study has demonstrated the potential of SELDI-TOF MS as a convenient biomarker discovery tool, which could be applied to studies of respiratory disease. Studies of plasma protein differences between asthmatics and healthy control subjects could generate novel biomarkers for diagnostic tests or provide tools for monitoring changes in asthma during conditions such as pregnancy.

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Table 1: Subject characteristics

	Asthmatic	Non-asthmatic
Maternal age (years)	26.9 ± 1.0 n=27	27.6 ± 0.9 n=23
Maternal weight in early pregnancy (kg)	78.9 ± 3.9 n=25	73.3 ± 3.3 n=19
Maternal height (cm)	163 (161 – 167) n=25	165 (163 – 173) n=19
Gravidity	2 (1 – 4) n=26	2 (1 – 3) n=22
Parity	1 (0 – 2) n=26	1 (0 – 2) n=23
Gestational age at delivery (weeks)	41 (39 – 41) n=27	40 (40 – 40) n=23
% of subjects using inhaled corticosteroids	52%	
% of subjects with mild asthma	<u>68%</u>	
% of subjects with moderate asthma	<u>9%</u>	
% of subjects with severe asthma	<u>23%</u>	
Average inhaled corticosteroid dose (µg/day)	200 (0 – 575) n=24	
Forced expiratory volume at 1 second (FEV ₁) (L)	2.85 ± 0.12 n=23	
Forced vital capacity (FVC) (L)	3.57 ± 0.12 n=23	

FEV₁:FVC Ratio (%)

80 ± 2

n=23

Values given are mean ± standard error of mean or median (interquartile range) or % of subjects within each group.

Table 2: Total number of peaks identified under each ProteinChip® and EAM condition at 18 and 30 weeks gestation

Total Number of peaks at 18 weeks gestation

ProteinChip® Condition	Mass range (laser intensity)	SPA	CHCA
SAX pH9	Low	34	26
	High	43	
WCX pH4	Low	31	46
	High	45	
IMAC-Cu ²⁺ pH7	Low	60	7
	High	33	

Total number of peaks at 30 weeks gestation

ProteinChip® Condition	Mass range (laser intensity)	SPA	CHCA
SAX pH9	Low	37	51
	High	46	
WCX pH4	Low	38	20
	High	41	
IMAC-Cu ²⁺ pH7	Low	66	8
	High	33	

SPA = sinapinic acid (energy absorbing molecule)

CHCA = α -cyano-4-hydroxycinnamic acid (energy absorbing molecule)

Table 3: Maternal plasma protein differences between asthmatic and non-asthmatic women at 18 weeks gestation (Category A peaks)

Chip conditions	Mass range (laser intensity)	Peak m/z	Peak height (asthmatic vs control)	P value	Fold Difference
WCX pH4 SPA	Low	3166	Lower	0.001	4.1
		6444	Higher	0.039	1.3
		6641	Higher	0.039	1.3
		8944	Lower	0.005	3.1
		13776	Higher	0.002	1.8
		13898	Higher	0.003	1.7
		14069	Higher	0.005	1.6
		22245	Higher	0.043	1.2
		28113	Higher	0.001	2.0
WCX pH4 SPA	High	22170	Lower	0.0002	1.5
		36641	Lower	0.001	1.8
		44359	Lower	0.0002	1.5
		51048	Lower	0.003	1.3
		58770	Higher	0.009	1.4
		99704	Lower	0.002	1.3
IMAC pH7 SPA	Low	3899	Higher	0.011	1.8
		4655	Higher	0.009	1.8
IMAC pH7 SPA	High	11744	Higher	0.0003	1.6

		11952	Higher	0.025	1.3
		14118	Higher	0.005	1.2
SAX pH9 SPA	Low	6444	Higher	0.005	1.6
		6642	Higher	0.003	1.6
		13898	Lower	0.0004	1.5
		15143	Higher	0.02	1.9
		28120	Higher	0.036	1.2
SAX pH9 SPA	High	13869	Lower	0.008	1.4
IMAC pH7 CHCA	Low	2029	Lower	0.036	1.7
		4138	Higher	0.002	2.7

SPA = sinapinic acid (energy absorbing molecule)

CHCA = α -cyano-4-hydroxycinnamic acid (energy absorbing molecule)

Table 4: Maternal plasma protein differences between asthmatic and non-asthmatic women at 30 weeks gestation (Category A peaks)

Chip conditions	Mass range (laser intensity)	Peak m/z	Peak height (asthmatic vs control)	P value	Fold Difference
IMAC pH7 SPA	Low	6391	Lower	0.01	1.9
		10083	Lower	0.01	2.4
		19915	Lower	0.028	1.7
IMAC pH7 SPA	High	19876	Lower	0.004	2.0
SAX pH9 SPA	Low	4077	Higher	0.01	3.3
		6444	Higher	0.009	1.6
		8708	Lower	0.001	1.3
		14062	Lower	0.001	1.5
		17401	Lower	0.039	1.4
SAX pH9 SPA	High	28090	Lower	0.048	1.3
		75623	Higher	0.003	1.4
		94332	Lower	0.012	1.2
IMAC pH7 CHCA	Low	1729	Higher	0.002	4.5
		1784	Lower	0.028	1.6
		1816	Higher	0.002	4.2
		2191	Lower	0.028	2.6
		3282	Lower	0.035	1.8
		22213	Lower	0.009	2.8

SPA = sinapinic acid (energy absorbing molecule)

CHCA = α -cyano-4-hydroxycinnamic acid (energy absorbing molecule)

Figure Legends

Figure 1: Asthma associated peptide in maternal plasma

Panel A shows the mean peak intensity $\pm$ SEM of a maternal plasma peak with m/z 6444 identified using a SAX chip, pH 9 with SPA as the EAM. * indicates $P < 0.05$ (t-test). Panel B shows a representative spectrum from a control and an asthmatic woman around the region 5-9 kDa.