Proteomic profiling of immunoglobulin A nephropathy in serum using magnetic bead based sample fractionation and matrix-assisted laser desorption/ionization-time of flight mass spectrometry

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Immunoglobulin A nephropathy (IgAN) is characterized by renal mesangial deposits of IgA. It can often lead to end stage renal disease (ESRD), and there is no known treatment proven to prevent ESRD in IgAN. It requires successful early detection along with adequate treatment for IgAN classification and diagnosis. Percutaneous renal biopsy is currently the only means to make a definite diagnosis. Unfortunately, biopsies are inconvenient, invasive and painful, and can result in some complications. Proteomics is a new, exciting, and largely unexplored area in IgAN. Preliminary studies have shown that this technique may provide a novel means of diagnosing IgAN, and it may have an additional value as a prognostic tool.[1] Serum proteome analysis has the potential to facilitate disease diagnosis and therapeutic monitoring, because serum is more easily accessible and widely collected compared to biopsy specimens that contains >10,000 different proteins and peptides.[2] Advances in mass spectrometry (MS) now could display thousands of peptides using trace quantity of serum.[3,4] The goal of this study was to obtain the serum peptide fingerprint in IgAN patients, to assess the utility of peptide profiling in a small sample population to identify potential biomarkers of IgAN, and to launch preliminary work for better understanding of the pathogenesis and early diagnosis of IgAN from an integrated perspective of proteomics.

We conducted this study in the Central Laboratory of the Department of Nephrology, 181 Hospital, Guilin, Guangxi Province, China from October 2009 to March 2010, using a novel platform called ClinPro Tools.[5] Thirty-three patients with biopsy confirmed IgAN (15-51 years old, 14 males, 19 females) were studied. Thirteen healthy volunteers (28-51 years old, 7 males, 6 females) served as normal controls, and 12 minimal change nephrotic syndrome (MCNS) (16-45 old, 9 males, 3 females), 10 membranous nephropathy (15-43 old, 9 males, 1 females), and 10 focal segmental glomerulosclerosis (FSGS) (22-59 years old, 8 males, 2 female) served as disease controls. This study was approved by the Ethics Committee of the institution. Data analysis was carried out using ClinPro Tools software 2.2 (Bruker Daltonik, Bremen, Germany). Statistical significance of different quantity of peptides was determined by means of Welch's t-tests. A *p*-value of <0.05 was considered statistically significant. Class prediction model was set up by Genetic Algorithm (GA). In our study, we successfully demonstrated that peak amount and peak area of peptide panels by magnetic

Table 1 - The significant differential peptide peaks between IgAN groups and other groups using genetic algorithm.

Group	Mass	Dave	PTTA	Ave 1	Ave 2	SD1	SD2	CV1	CV2
IgAN versus N	7761.30	14.61	<0.000001	23.85	9.25	6.77	1.90	28.37	20.52
	5902.66	22.20	<0.000001	41.15	18.96	19.02	5.51	36.17	29.07
	4053.48	9.33	<0.000001	13.95	23.28	9.43	6.35	27.58	27.29
	9281.79	8.46	<0.000001	33.46	25.01	10.24	6.99	30.62	27.94
	6431.92	2.16	0.00041	1.94	5.10	0.66	3.35	34.04	51.66
IgAN versus MCNS	7761.50	17.94	<0.000001	22.56	4.62	6.37	2.57	28.24	35.66
	4963.02	11.92	<0.000001	15.62	3.70	10.25	3.20	25.65	26.53
	9281.95	14.36	<0.000001	32.19	17.83	9.87	8.93	20.67	30.08
	5915.93	13.14	0.0000386	9.94	23.07	4.50	11.50	45.31	49.85
	6628.87	5.05	0.00266	4.25	12.30	2.17	6.75	51.05	52.63
IgAN versus MN	7761.40	15.78	<0.000001	22.56	6.78	6.37	2.43	28.24	35.86
	4643.29	10.41	<0.000001	18.77	8.36	5.93	2.84	31.58	33.95
	9281.87	16.30	<0.000001	32.19	15.89	9.87	5.58	30.67	35.12
	6674.38	11.86	0.00189	4.25	16.11	2.17	10.66	41.05	46.18
	6430.65	6.54	0.00255	1.40	7.94	0.69	6.23	39.41	48.50
	4209.49	13.88	0.00289	47.69	33.81	18.32	13.11	38.41	38.79
IgAN versus FSGS	7761.36	17.79	<0.000001	22.56	4.77	6.37	1.54	28.24	32.30
	9281.83	18.34	<0.000001	32.19	13.85	9.87	5.68	20.67	30.98
	5902.71	18.76	0.00000341	38.87	20.11	18.04	10.15	36.40	40.47
	6642.94	3.76	0.0000477	1.83	5.59	0.66	2.43	36.26	43.53
	6428.83	3.91	0.00509	4.25	8.16	2.17	4.31	41.05	42.78

IgAN - immunoglobulin A nephropathy, N - normal, MCNS - minimal change nephrotic syndrome, MN - membranous nephropathy, FSGS - focal segmental glomerulosclerosis, mass - m/z value, Dave - difference between the maximal and the minimal average peak area/intensity of all classes, PTTA - *p*-value of t-test, average - peak area/intensity average of class, SD - standard deviation of the peak area/intensity average of class, CV - coefficient of variation in % of class, 1 - the former class of groups, 2 - the latter class of groups

beads based weak cation exchange were significant and useful parameters for diagnosis. The resulting multiple spectrum profiles were analyzed and compared to obtain specifically disease-related peptides. Between IgAN and normal controls, 58 peptides were significant, 3 were highly elevated, and 2 were highly degraded. Between IgAN and MCNS group, 64 peptides were significant, 3 were highly elevated, and 2 were highly degraded. Between IgAN and MN group, 29 peptides were significant, 4 were highly elevated, and 2 were highly degraded. Between IgAN and FSGS group, 29 peptides were significant, 3 were highly elevated, and 2 were highly degraded. The data of elevated and degraded peptide peaks are shown in **Table 1**. The raw data were then analyzed using ClinPro Tools software 2.2, and GA processing were used to classify sample, and establish diagnostic model. With the established model, the cross validation of IgAN distinguished from normal controls of the groups were: MCNS - 100%, MN - 98.53%, and FSGS - 100%. The recognition capabilities were 100%. Based on this study, valuable differential peptides were identified and potential biomarkers for IgAN diagnosis were obtained. With established diagnostic model, IgAN could be effectively separated from controls, which demonstrated that GA processing would be an effective method to set up diagnostic model with high sensitivity and specificity. The established models may apply as alternative means to clinic diagnosis of IgAN. A limitation of this study was the small population, and a larger sized study is needed in further research.

In conclusion, our study established a serum peptide fingerprint model for diagnosis of IgAN using GA processing. This preliminary study demonstrated that serum peptide fingerprint could provide a new way for further study in diagnosing IgAN.

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References

1. Haubitz M, Wittke S, Weissinger EM, Walden M, Rupprecht HD, Floege J, et al. Urine protein patterns can serve as diagnostic tools in patients with IgA nephropathy. ***Kidney Int*** 2005; 67: 2313-2320.
2. Thadikkaran L, Siegenthaler MA, Crettaz D, Queloz PA, Schneider P, Tissot JD. Recent advances in blood-related proteomics. ***Proteomics*** 2005; 5: 3019-3034.
3. Koomen JM, Li D, Xiao LC, Liu TC, Coombes KR, Abbruzzese J, et al. Direct tandem mass spectrometry reveals limitations in protein profiling experiments for plasma biomarker discovery. ***J Proteome Res*** 2005; 4: 972-981.
4. Villanueva J, Philip J, Entenberg D, Chaparro CA, Tanwar MK, Holland EC, et al. Serum peptide profiling by magnetic particle-assisted, automated sample processing and MALDI-TOF mass spectrometry. ***Anal Chem*** 2004; 76: 1560-1570.
5. Ketterlinus R, Hsieh SY, Teng SH, Lee H, Pusch W. Fishing for biomarkers: analyzing mass spectrometry data with the new ClinProt Tools software. ***Biotechniques*** 2005; 38: 37-40.