

Urotensin 2 and Retinoic Acid Receptor Alpha (RARA) Gene Expression in IgA Nephropathy

Keng Thye Woo,^{1FRACP} Yeow Kok Lau,^{1PhD} Yi Zhao,^{2PhD} Kim Yoong Puong,^{2PhD, MSc} Hwee Boon Tan,^{1BSc},
Stephanie Fook-Chong,^{2MSc} Kok Seng Wong,^{1FRCP} Choong Meng Chan^{1FRCP}

Abstract

Introduction: IgA nephropathy is a disease where the pathogenesis is still poorly understood. Deoxyribonucleic acid (DNA) microarray technique allows tens of thousands of gene expressions to be examined at the same time. Commercial availability of microarray genechips has made this powerful tool accessible for wider utilisation in the study of diseases. **Materials and Methods:** Seven patients with IgA nephropathy, 6 with *minimal change nephrotic syndrome* (MCNS) as patient controls and 7 normal healthy subjects were screened for the differential expression of genes, genome-wide. The Human Genome U133 Plus 2.0 Arrays (Affymetrix, USA) were used to quantitate the differential expression of 38,500 well-characterised human genes. **Results:** A total of 7761 gene expressions were identified that have an IgAN/Normal gene expression ratio of 0.06-fold to 5.58-fold. About 35% of the altered gene expressions have no gene title or just a hypothetical protein label such as FLJ30679. Most of the remaining 65% are identified proteins where their importance to IgAN is not immediately apparent at this time. Among the 30 most upregulated and 30 most downregulated genes are Urotensin 2 (upregulated 3.09-fold, $P < 0.05$) and Fatty-acid binding protein 6 (downregulated to 0.12-fold, $P < 0.05$). Retinoic acid receptor alpha (vitamin A receptor) was also found downregulated to 0.41-fold ($P < 0.005$). Taqman real-time polymerase chain reaction (PCR) for urotensin 2 and retinoic acid receptor alpha (RARA) were performed on 20 patients with IgA nephropathy and 11 with *Minimal Change Disease* and the data correlated with various clinical indices. **Conclusions:** The findings suggest that there may be a therapeutic role for retinoic acid receptor alpha (RARA) in IgA nephropathy and a clinical monitoring role for Urotensin 2 in *Minimal Change Disease*.

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Introduction

IgA nephropathy (IgAN) is the most common primary glomerulonephritis in Singapore¹ and in many parts of the world,² contributing significantly to the pool of end-stage renal failure patients annually. Despite more than 3 decades of research, the pathogenesis of the disease is still poorly understood. DNA microarray technique allows tens of thousands of gene expressions genome-wide to be examined together simultaneously. This advance in gene expression profiling has given rise to immense potential for the characterisation and prognostication of disease, and for analysing the complex biological processes involved which would allow a better understanding of the molecular basis of diseases.^{3,4} With the aid of bioinformatic tools, sophisticated questions in research can also be answered.

Now the commercial availability of microarray genechips has made this powerful tool more accessible for wider utilisation in the study of diseases. In this study, we analyse the genome-wide gene expressions in 7 IgAN patients, 6 with *Minimal Change Disease* as patient controls and 7 normal subjects by using the GeneChip Human Genome U133 Plus 2.0 Arrays from Affymetrix. Individual specific gene expression of interest was confirmed using Taqman real-time PCR method.

Materials and Methods

Patients

Seven patients with biopsy proven IgAN, 6 with *Minimal Change Disease* (Non-IgAN patient controls) and 7 healthy controls were investigated for genome-

¹ Department of Renal Medicine, Singapore General Hospital, Singapore

² Department of Clinical Research, Singapore General Hospital, Singapore

Address for Correspondence: Prof Keng Thye Woo, Department of Renal Medicine, Singapore General Hospital, Outram Road, Singapore 169608.

Email: woo.keng.thye@sgh.com.sg

wide gene expression. Subsequently, individual specific gene expression of interest was confirmed by Taqman real-time PCR technique. All subjects studied were ethnic Chinese. Prior informed consent was obtained from each subject before a sample of 2 ml EDTA-blood was taken. In the second part of the study using Taqman real time PCR studies, 20 patients with IgAN and 11 with *Minimal Change Disease* had Urotensin 2 (URT2) and retinoic acid receptor alpha (RARA) assay performed. The patients had their blood pressure (BP) recorded at the time of sampling for URT2 and RARA as well as serum creatinine and proteinuria. Patients with IgAN were treated with losartan 100 mg a day for the reduction of proteinuria and those with hypertension were treated with amlodipine and/ or atenolol. Patients with *Minimal Change Disease* were all in remission for the nephrotic syndrome and were on small doses of maintenance prednisolone. Those with hypertension were treated with amlodipine and/or atenolol. Three years after the study, all patients had their BP, serum creatinine and proteinuria measured again to assess for correlation with URT2 and RARA to determine whether URT2 and RARA could be useful predictors for disease progression.

Genome-wide Gene Expression Analysis

Total leukocyte ribonucleic acid (RNA) was isolated from each specimen and biotinylated cRNA targets were prepared with 5 ul of total RNA according to manufacturer's protocols (Affymetrix, Santa Clara, CA). Targets were hybridised to GeneChip Human Genome U133 Plus 2.0 Arrays for 16 hours. The hybridised arrays were washed and stained with streptavidin/phycoerythrin (SAPE) conjugate and antistreptavidin antibody in Affymetric fluidic station 450 and scanned with GeneChip scanner 3000. Signals were analysed with the GeneChip operating system (GCOS) software. Detection ('present', 'absent' or 'marginal'), change ('increased', 'decreased' or 'no change') and fold change status (-log2) between the signals generated from patients' specimens compared to controls were analysed with Affymetrix data mining tool. The numerical values in Table 1 represent the signals of fluorescence intensity.

Taqman Real-time Quantitative PCR for Confirmation of Single Specific Gene Expression

For every single gene expression test, a pair of primers will be used and a human gene will be an endogenous reference. The sample from normal healthy persons will be used as controls for gene expression by real-time PCR.

SYBR Green PCR Master Mix (PE Biosystem) will be used for real-time PCR amplification. For each reaction, cDNA will be taken as an initial template. Thermal cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Real-time PCR was performed using 7700-sequence

Table 1. Clinical and Gene Expression Profile of Patients with IgAN, non-IgAN, Normal Controls

	IgAN	non-IgAN	Controls
Number (n)	7	6	7
Sex, m : f	5 : 2	4 : 3	3 : 3
Age, years	52 ± 8*	40 ± 13	38 ± 7*
Serum Creatinine, µmol/l	173 ± 75†	81 ± 35†	-
Proteinuria, mg/day	673 ± 728	293 ± 424	-
Urotensin 2, arbitrary units (au)	1761 ± 1124†	816 ± 472	569 ± 405†
Fatty acid binding protein 6, au	10 ± 6†	15 ± 8†	84 ± 65†
Retinoic acid receptor alpha, au	231 ± 77‡	339 ± 200	561 ± 227 ‡

*P < 0.01 †P < 0.05 ‡P < 0.01

detector system (Applied Biosystem, USA). The gene expression level in unknown samples will be quantitated using the comparative C_T method ($\Delta\Delta C_T$ method). The method is based on the inverse exponential relationship that exists between the initial quantity of the target copy number in the reaction and corresponding C_T value. Following the arithmetic formulae, the amount of target, normalised to the endogenous reference and relative to a calibrator, is given by $2^{-\Delta\Delta C_T}$, Where $\Delta\Delta C_T = \Delta C_{T, \text{SMN}} - \Delta C_{T, \text{normal}}$ and each $\Delta C_T = C_{T, \text{target}} - C_{T, \text{reference}}$. The numerical values (units) for Taqman RT-PCR for both URT2 and RARA denote the number of folds change compared with the value for Normal Healthy Control which is calibrated to 1 fold. For the Patient with *Minimal Change Disease* in the Control Group, Taqman RT-PCR was not performed as there was not enough blood sample left to do the test. Therefore only URT2 was performed for the Patient Control Group (Tables 2 and 3).

Genes with significantly altered expression levels were further investigated with a search in the PUBMED databases for properties and functions that may be relevant to the pathophysiology of IgAN. Using Taqman real-time PCR, the values (number of folds change) for URT2 and RARA in 20 patients with IgAN and 11 patients with *Minimal Change Disease* were studied for correlation with clinical indices to assess their roles as possible clinical markers.

Statistics

Results are expressed as Mean and Standard Deviation for IgAN patients and controls. Student's t-test was used to detect significance of differences between the means in the various comparison groups. Pearson's correlation coefficient was employed to test for correlation between various clinical indices with URT2 and RARA. Multiple linear regression (MLR) of each clinical index was also carried out to verify its association with URT2, adjusting for age, sex and other confounding factors. Similarly, multiple linear regression of each clinical index was carried out to

Table 2. Clinical Profile and Taqman RT-PCR Data of Urotensin 2 and Retinoic Acid Receptor Alpha in IgA Nephritis and Non-IgA Nephritis

Renal diagnosis	Sex	Age	Systolic BP, mmHg (Initial)	Diastolic BP, mmHg (Initial)	Serum Creatinine, mg/l (Initial)	Proteinuria, gm/day (Initial)	Systolic BP, mmHg (After)	Diastolic BP, mmHg (After)	Serum Creatinine, mg/l (After)	Proteinuria, gm/day (After)	Urotensin 2 (units)	Retinoic Acid Receptor Alpha (RARA) (units)
IgA Nephritis Case												
1	F	48	120	80	99	2.50	120	80	109	1.98	1.32	1.94
2	F	48	130	80	67	0.10	120	80	76	0.20	0.89	
3	M	59	150	90	360	2.20	140	80	436	2.60	1.15	0.96
4	M	46	130	80	211	0.30	130	70	199	3.10	0.35	1.84
5	M	49	160	100	117	6.30	140	85	122	0.30	2.56	0.95
6	F	41	150	100	74	3.00	130	90	66	0.40	2.46	0.55
7	F	41	130	80	83	3.20	110	70	132	2.10	0.44	1.09
8	M	47	140	90	71	1.10	150	90	65	0.50	1.42	1.30
9	F	45	120	60	202	0.80	110	70	176	0.20	0.97	0.95
10	M	43	150	100	106	2.40	130	80	101	3.90	2.34	
11	F	34	120	80	88	4.00	120	80	139	0.20	0.83	1.34
12	F	48	160	90	115	4.20	130	75	435	1.60	2.23	1.53
13	M	59	110	60	116	0.10	120	80	102	0.10	0.39	1.57
14	M	43	130	80	201	1.37	130	85	221	1.80	1.44	0.59
15	F	25	150	100	78	2.60	120	70	97	1.70	4.38	0.42
16	F	52	150	90	97	2.90	140	80	165	4.00	5.74	0.65
17	M	24	130	80	120	2.50	130	90	277	1.40	1.25	0.72
18	F	44	120	60	110	2.00	130	70	84	2.90	1.09	1.68
19	M	65	150	100	133	4.30	120	80	222	1.60	7.34	1.13
20	F	54	140	90	53	6.60	130	70	63	0.80	3.18	1.57
Mean		46	137	85	121	2.99	128	79	164	1.57	2.09	1.15
± SD		10	15	13	68	2.21	10	7	110	1.25	1.85	0.47
Non-IgA Nephritis Case												
1	M	35	120	70	236	0.60	130	80	411	0.60	1.42	
2	F	56	140	100	126	0.90	120	75	356	4.10	2.67	
3	M	51	140	80	111	2.20	150	90	119	0.50	0.77	
4	F	41	110	60	58	0.10	120	70	66	0.30	0.20	
5	F	34	120	80	54	0.10	130	80	49	0.10	0.70	
6	M	19	110	70	76	0.10	120	70	96	0.10	0.24	
7	M	39	120	70	86	0.36	110	60	79	0.10	0.61	
8	F	52	150	80	71	0.10	120	75	94	0.10	1.19	
9	F	47	130	80	68	0.20	115	70	73	0.10	0.63	
10	F	39	120	70	56	0.80	120	80	59	0.30	1.37	
11	M	55	140	80	134	0.20	130	75	152	0.20	0.87	
Mean		43	127	76	98	1.51	124	75	141	0.59	0.97	
± SD		11	13	10	54	3.24	11	8	124	1.18	0.69	

Table 3. Comparison of Clinical Profile & Gene expression (Taqman RT-PCR) for Urotensin 2 and Retinoic Acid Receptor Alpha in IgA Nephritis and *Minimal Change Disease* (Non-IgA Nephritis)

	IgAN	Non-IgAN	P value
Number of cases	20	11	
Sex, m : f	9 : 11	5 : 6	n.s.
Age, years	46 ± 10	43 ± 11	n.s.
Systolic BP, mmHg(Initial)	137 ± 15	127 ± 13	n.s.
Diastolic BP, mmHg (Initial)	85 ± 13	76 ± 10	n.s.
Serum Creatinine, µmol/l(Initial)	121 ± 68	98 ± 54	n.s.
Proteinuria, gm/day(Initial)	2.99 ± 2.21	1.51 ± 3.24	n.s.
Systolic BP, mmHg (After)	128 ± 10	124 ± 11	n.s.
Diastolic BP, mmHg (After)	79 ± 7	75 ± 8	n.s.
Serum Creatinine, µmol/l(After)	164 ± 110	141 ± 123	n.s.
Proteinuria, gm/day(After)	1.57 ± 1.25	0.59 ± 1.18	P < 0.05
Urotensin 2 (units)	2.09 ± 1.85	0.97 ± 0.69	P < 0.05
Retinoic acid receptor alpha (units)	1.15 ± 0.47	not done	-

Data expressed as Mean ± SD; n.s. = not significant at the 0.05 level (2-tailed)

verify its association with RARA, adjusting for age, sex and other confounding factors. For instance, a multiple linear regression of systolic blood pressure at baseline on URT2, adjusting for age, sex, baseline serum creatinine and baseline total urine protein was done following bivariate correlation analysis of systolic blood pressure with URT2.

Results

A total of 7761 gene expressions were identified that had an IgAN/Normal gene expression ratio of 0.06-fold to 5.58-fold (213 were upregulated with ratio ≥ 2.0 -fold and 244 were downregulated with ratio ≤ 0.2 -fold). About 35% of the altered gene expressions had no gene title or just a hypothetical protein label such as FLJ30679. Most of the remaining 65% were identified proteins such as the most upregulated gene. Nevertheless, initial analysis of the first 30 most upregulated (Table 4) and 30 most downregulated (Table 5) gene expressions have produced some interesting findings which may be relevant to IgAN.

Urotensin 2 (URT2) (Table 4, No.30) was upregulated 3.09-fold (1762 ± 1125 vs 570 ± 406 arbitrary units, $P < 0.05$) in patients with IgAN compared to patient and normal controls. Fatty acid binding protein 6 (FABP6) (Table 5, No. 30) was downregulated to 0.12-fold (10 ± 6 vs 84 ± 64 arbitrary units, $P < 0.05$) in patients with IgAN compared to patient controls and normal controls.

Table 1 shows the gene expression profile of patients with IgAN, Non-IgAN (*Minimal Change Disease*) and Normal Controls. Retinoic Acid Receptor Alpha (RARA)

gene expression was found to be downregulated to 0.41-fold (231 ± 77 vs 561 ± 227 units, $P < 0.005$) in patients with IgAN compared to patient and normal controls. There was an age difference between the Normal Controls and the IgA nephritis patients and this may have an influence on the gene expression results. However there was no age difference between the Patient Controls (*Minimal Change Disease*) and the IgA nephritic group.

Table 2 shows the Clinical Profile for patients with IgAN (n = 20) and *Minimal Change Disease* (n = 11). Table 3 compares the data between patients with IgAN and non-IgAN. Table 6 shows the correlation coefficients between the URT2 and RARA gene expression with the clinical indices in patients with IgAN. Among patients with IgAN, both systolic (137 ± 15 mmHg) ($r = 0.60$, $P < 0.005$) and diastolic BP (84 ± 13) ($r = 0.61$, $P < 0.004$) were positively correlated with URT2 levels. Figures 1 and 2 show the correlation between URT2 and systolic and diastolic BP in IgAN. In multiple linear regression of systolic BP and diastolic BP, URT2 was found significant even after adjustment for confounding factors ($\beta = 4.58$, $P = 0.006$ and $\beta = 4.67$, $P = 0.001$ for MLR of systolic BP and diastolic BP respectively).

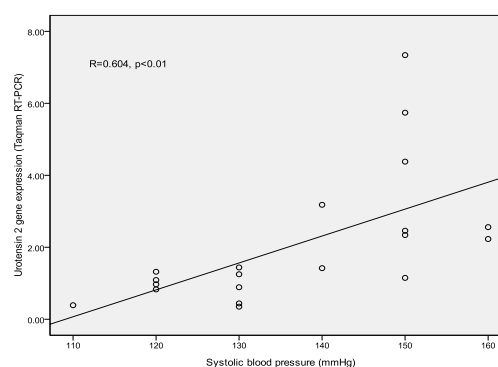


Figure 1: Correlation between Urotensin 2 gene expression (Taqman RT-PCR) and Systolic blood pressure in IgA Nephritis (n=20)

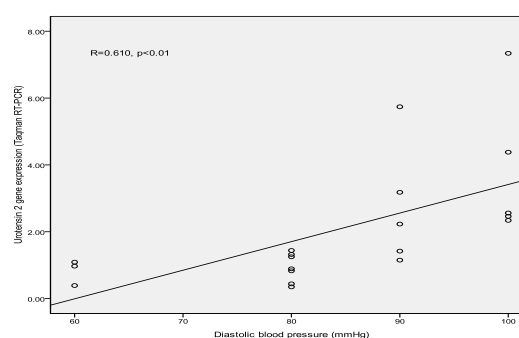


Figure 2: Correlation between Urotensin 2 gene expression (Taqman RT-PCR) and Diastolic blood pressure in IgA Nephritis (n=20)

Table 4. Genome-wide Gene Expression in IgA Nephropathy: 30 Most Upregulated

No.	Expression (x Normal)	Prob Set ID	Gene Title	Gene Symbol
1.	5.58	1569362_at	Activated leucocyte cel adhesion molecule	ALCAM
2.	5.26	236380_at	-	-
3.	5.14	218029_at	Hypothetical protein FLJ13725	FLJ13725
4.	5.04	206473_at	Membrane-bound transcription factor protease, site 2	MBTPS2
5.	4.92	230442_at	Hypothetical protein FLJ12998	FLJ12998
6.	4.49	236407_at	-	-
7.	4.06	220617_s_at	Zing finger protein 532	ZNF532
8.	4.05	201406_at	Ribosomal protein L36a	RPL36A
9.	3.90	239546_at	PQ loop repeat containing 2	PQLC2
10.	3.88	1564467_at	Hyothetical protein FLJ13305	FLJ13305
11.	3.88	221906_at	Thioredoxin reductase 3	TXNRD3
12.	3.75	200061_s_at	Ribosomal protein S24	RPS24
13.	3.64	202904_s_at	LSM5 homolog, U6 small nuclear RNA associated	LSM5
14.	3.62	228388_at	Nuclear factor of kappa light polypeptide gene enhancer	NFKBIB
15.	3.53	205849_s_at	Ubiquinol-cytochrome c reductase binding protein	UQCRB
16.	3.50	200026_at	Ribosomal protein L34	RPL34
17.	3.47	212169_at	FK506 binding protein 9	FKBP9
18.	3.45	209804_at	DNA cross-link repair	DCLRE1A
19.	3.38	1553311_at	Hyothetical protein FLJ33860	FLJ33860
20.	3.37	227908_at	KIAA1171 protein	KIAA1171
21.	3.36	1565755_at	-	-
22.	3.33	236849_at	-	-
23.	3.28	220260_at	Hypothetical protein FLJ11082	FLJ11082
24.	3.28	222229_x_at	Ribosomal protein L26	RPL26
25.	3.22	1569596_at	-	-
26.	3.17	1552705_at	Dual specificity phosphatase 19	DUSP19
27.	3.15	236595_at	-	-
28.	3.11	204451_at	Frizzled homolog 1 (Drosophila)	FZD1
29.	3.10	207877_s_at	Nuclear VCP-like	NVL
30.	3.09	220784_s_at	Urotensin 2	UTS2

Serum creatinine and urinary protein levels were not correlated with URT2. There was no correlation between serum creatinine and proteinuria with URT2, before and 3 years after the study. For *Minimal Change Disease*, there was a positive correlation between URT2 and the diastolic BP (76 ± 10 mmHg) ($r = 0.73$, $P < 0.01$) at time of study as well as the serum creatinine (141 ± 124 microMol/l) ($r = 0.71$, $P < 0.02$) and proteinuria (0.59 ± 1.18 gm/day) ($r = 0.84$, $P < 0.001$) 3 years after the study. There was no correlation between URT2 and systolic BP.

Table 6 shows the correlation coefficients and P values between URT2 and RARA and the Clinical and Laboratory indices in patients with IgAN. There was only a correlation between URT2 and the initial systolic and diastolic BP. For RARA, no correlation was found with BP, serum creatinine and proteinuria.

Discussion

A total of 7761 gene expressions were identified with

Table 5. Genome-wide Gene Expression in IgA Nephropathy: 30 Most Downregulated

No.	Expression (x Normal)	Prob Set ID	Gene Title	Gene Symbol
1.	0.06	1560540_x_at	Eukaryotic translation initiation factor 4E binding protein 3	ANKHD1
2.	0.06	207415_at	Phospholipase A2 receptor 1, 180kDa	PLA2R1
3.	0.06	244880_at	-	-
4.	0.07	1553654_at	Synaptotagmin XIV	SYT14
5.	0.07	1567076_at	-	-
6.	0.07	243164_s_at	-	-
7.	0.08	1556876_s_at Pseudogene	TPTE,PTENhomologInositolLipidPhosphatase	TPTEps1
8.	0.08	1559545_at	SNRPN upstream reading frame	SNRPN
9.	0.06	232318_s_at	Hypothetical protein LOC121838	LOC121838
10.	0.08	234039_at	TPR domain, ankyrin-repeat and coiled-coil -containing	TANC
11.	0.08	242348_at	TAF4 protein	TAF4
12.	0.09	230067_at	-	-
13.	0.10	1555313_a_at	MCF.2 cell line derived transforming sequence	MCF2
14.	0.10	228388_at	CDNA FLJ440922 fis, clone TEST14041985	-
15.	0.10	1566424_at	-	-
16.	0.10	1567280_at	-	-
17.	0.10	207333_at	Neuromedin B receptor	NMBR
18.	0.10	207779_at	-	-
19.	0.10	210885_s_at	Tripartite motif-containing 15	RIM15
20.	0.10	216772_at	-	-
21.	0.10	236421_at	FLJ45235 protein	FLJ45235
22.	0.10	237070_at	Transient receptor potential cation channel, subfam,M,memb1	TRPM1
23.	0.10	243681_at	SH3 and multiple ankyrin repeat domains 2	SHANK2
24.	0.11	1554506_x_at	N-acetylated alpha-linked acidic dipeptidase 2	NAALAD2
25.	0.11	1565817_at	-	-
26.	0.11	206265_s_at	glycosylphosphatidylinositol specific phospholipase D1	GPLD1
27.	0.11	216045_at	KIAA0565 gene product	KIAA0565
28.	0.11	236848_s_at	Testis expressed sequence 13A	TEX13A
29.	0.12	1556364_at	CDNA FLJ25194 fis, clone REC04095	-
30.	0.12	1566126_at	Fatty acid binding protein 6, ileal (gastrotropin)	FABP6

about 35% of the altered gene expressions having no gene title or just a hypothetical protein label while most of the remaining 65% were identified proteins such as the most upregulated gene, activated leucocyte cell adhesion molecule which has been implicated in tumorigenesis⁵ but their importance and relevance to IgAN is not immediately apparent at this time.

The data we have at hand show that patients with IgAN have upregulation of Urotensin 2 (URT2) gene expression and downregulation of fatty acid binding protein 6 (FABP6) as well as retinoic acid receptor alpha (RARA) gene expression. Urotensin 2 (URT2) was upregulated 3.09-fold in IgAN patients compared to patient and normal controls.

Table 6. Pearson's Correlation between Urotensin 2 and Retinoic Acid Receptor Alpha gene expression (Taqman RT-PCR) and Clinical Indices in IgA Nephritis

	Urotensin 2 (n = 20)		Retinoic acid receptor alpha (n = 18)	
	R	P value	R	P value
Age (Years)	0.251	n.s.	0.371	n.s.
Systolic BP, mmHg (Initial)	0.604	0.005	-0.425	n.s.
Diastolic BP, mmHg (Initial)	0.610	0.004	-0.413	n.s.
Serum Creatinine, $\mu\text{mol/l}$ (Initial)	-0.227	n.s.	0.193	n.s.
Proteinuria, gm/day (Initial)	0.288	n.s.	-0.079	n.s.
Systolic BP, mmHg (After)	0.133	n.s.	-0.084	n.s.
Diastolic BP, mmHg (After)	0.033	n.s.	-0.354	n.s.
Serum Creatinine, $\mu\text{mol/l}$ (After)	0.008	n.s.	-0.073	n.s.
Proteinuria, gm/day (After)	0.229	n.s.	0.041	n.s.

n.s. = not significant at the 0.05 level (2-tailed)

Fatty acid binding protein (FABP) is an essential component in the bile acid transport system in the rabbit ileum.⁶ The downregulation of FABP suggests that disturbance in lipid metabolism may have a role in the pathogenesis of IgAN in man and this could offer a potential new avenue for IgA research. For now, the link between fatty acid and IgA nephritis is still unknown.

URT2 is the most potent mammalian vasoconstrictor identified. Its receptor UT, exhibits increased expression in cardiac tissue and in plasma of patients with congestive heart failure. It is believed that URT2 plays a hypertrophic role in cardiac hypertrophic remodelling but does not affect cell proliferation or apoptosis.⁷ Apart from its potent vasoconstrictor actions, URT2 has also been found to have trophic and profibrotic effects. Recently, some investigators⁸ have examined the expression and localisation of URT2 and UT in renal biopsy tissue samples from patients with diabetic nephropathy using quantitative RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) and determined the intrarenal distribution of their peptides by means of immunohistochemistry.

Diabetic nephropathy has been reported to have a dramatic overexpression of URT2, implicating this vasoactive peptide as a possible novel factor in the pathogenesis of diabetic nephropathy.⁸ In human diabetic tissue, gene expression of URT2 and UT were increased 45 and almost 2000 fold in comparison to nephrectomised tissue ($P < 0.0001$). The authors⁸ concluded that in the context of its known biological actions, the dramatic overexpression of URT2 and UT implicate the vasoactive peptide as having a role as a possible novel factor in the pathogenesis of diabetic

nephropathy. Balat et al⁹ reported that plasma URT2 levels were decreased in children with *Minimal Change Nephrotic Syndrome*, compared to those during remission ($P < 0.05$) whereas the urinary URT2 levels were increased during relapse compared to those during remission ($P < 0.05$). It was concluded that the higher urinary URT2 levels during relapse could be the result of heavy proteinuria.

In our study, URT2 was upregulated 3.09-fold in IgAN patients compared to patient and normal controls. Taqman RT-PCR data showed that URT2 values were positively correlated with the level of both systolic and diastolic BP in patients with IgAN. These findings suggest that URT2 is involved in the pathogenesis of hypertension in IgAN. IgAN is the commonest form of glomerulonephritis in Singapore and hypertension is a well documented poor prognostic index.^{1,2} The patients in this study had their BP as well as blood samples for genomic studies collected at the same time, hence a temporal relationship between the raised BP and the elevated URT2 levels, implying a role for URT2 as a marker of vascular activity. However, based on the absence of correlation between URT2, serum creatinine and proteinuria in patients with IgAN, our data do not support URT2 as a predictive or prognostic index in IgAN. For *Minimal Change Disease* (Patient Control Group), there was a positive correlation between URT2 and diastolic BP suggesting a role for vascular activity for URT2 as in IgAN. We also found that URT2 was correlated with the serum creatinine and proteinuria of patients with *Minimal Change Disease* despite the fact that these patients were studied when they were in remission in the absence of renal failure or nephrotic syndrome and while on small doses of maintenance prednisolone. This is in agreement with the findings of Balat et al⁹ who also found low levels of URT2 for children with *Minimal Change Disease* while in remission. In our cases with *Minimal Change Nephrotic Syndrome*, they had low levels of protein as they were in remission and these levels correlated with the low URT2 levels.

Retinoids (vitamin A derivatives) are signalling molecules important in regulating cell proliferation, differentiation and apoptosis. Retinoids act through receptors such as retinoic acid receptor alpha (RARA) which are ligand-dependent transcription factors in the nucleus. There is an overexpression of RARA in intrinsic ageing of human skin¹⁰ and reduced levels of RARA per gram of brown adipose tissue in mice chronically fed a vitamin A-deficient diet.¹¹

Our finding of downregulating of mRNA expression for RARA in IgAN may suggest a new therapeutic approach for IgAN, a disease where hitherto there is no known therapy. Shaier's model of Thy-GN mice¹² is akin to the human disease of IgAN since the Thy-GN mice have Mesangial Proliferative Glomerulonephritis (GN) like human IgAN

where there is also Mesangial Proliferative GN. In Shaier's report,¹² Thy-GN mice treated with RARA agonist had reduced proteinuria and normalisation of blood pressure. We postulate that RARA specific retinoids may provide a therapeutic approach to the therapy of IgAN. RARA belongs to the steroid hormone superfamily of nuclear receptor proteins which exert their effects by binding specific DNA response elements, thus regulating gene expression in target cells.^{10,11} Regulating or normalising RARA mRNA expression in patients with IgAN may thus offer a novel therapeutic approach in terms of gene therapy. This is especially relevant to IgAN which has hitherto no known specific therapy. However, apart from finding low levels of RARA in patients with IgAN, we subsequently found no correlation between RARA and any of the clinical indices studied using Taqman RT-PCR.

Microarray technology, together with the aid of bioinformatics softwares, is now widely employed for rapid investigation into the complex biology in health and in disease.¹³⁻¹⁶ In IgA nephropathy, Preston et al¹⁷ had shown that gene expression profiles of circulating leukocytes correlate with renal disease activity in IgA nephropathy by combining microarray technology with clustering statistics. Genome-wide scan technique had also been applied in a mouse model of IgAN with rapid results in the identification of a susceptibility locus in chromosome 10.¹⁸ In this study, we found that a literature search on genes with altered expression can also be very useful in providing interesting information on novel biological processes and pathways which may shed new light on the pathophysiology of IgAN.

To summarise, our data suggest that URT2 is a marker of vascular disease in IgAN as patients with raised BP (systolic BP and diastolic BP) tend to have higher levels of URT2. URT2 levels were low in patients with *Minimal Change Nephrotic Syndrome* in remission. This is consistent with the findings of Balat et al.⁹ The diastolic BP and proteinuria were also correlated with URT2 in *Minimal Change Disease*. Our data showed that patients with IgAN had downregulation of RARA expression. Accordingly, based on the studies by Shaier et al¹² where Thy-GN rats with Mesangial Proliferative GN were reported to respond to RARA agonist with the reduction of proteinuria and normalisation of BP, there may be a role for clinical trials with RARA specific retinoids in patients with IgAN since IgAN is also a Mesangial Proliferative GN like Thy-GN.

Conclusion

Thousands of genes remain to be explored. Furthermore, with the gradual identification of the many untitled and hypothetical proteins, the list of identified genes may be repeatedly visited for more information. Assayed simultaneously on the same microarrays, these genes are

linked in expression, be it up- or down-regulation. Such linkage information on gene-gene interactions genome-wide may be integrated into a panoramic view of disease pathways to explain the origin and development of IgAN. The present preliminary analysis of genome-wide gene expressions has implicated Urotensin 2 upregulation and retinoic acid receptor alpha downregulation in the pathogenesis of IgA nephropathy. These may be relevant targets for further research and perhaps development of new drug therapy. Based on our preliminary data, perhaps a larger study is warranted with more observations based on serial lab and clinical data rather than a cross-sectional design which has its limitations.

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