RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2010.36.130

ScienceAsia 36 (2010): **130–136**

Association of toll-like receptor 4 gene polymorphisms with primary membranous nephropathy in a high prevalence renal disease area in Taiwan

Shih-Yin Chen[a,b], **Wen-Chi Chen**[a,b], **Yung-Hsiang Chen**[b], **Cheng-Hsu Chen**[c], **Yu-Chuen Huang**[a,b], **Po-Hsun Huang**[d], **Yi-Wen Lin**[b], **Huey-Yi Chen**[a,b], **Wen-Ling Liao**[b], **Fuu-Jen Tsai**[a,b,*]

[a] Genetics Centre, Department of Medical Research, Department of Urology, Department of Obstetrics and Gynecology, and Department of Pediatrics, China Medical University Hospital, Taichung, Taiwan

[b] Graduate Institute of Integrated Medicine, Graduate Institute of Acupuncture Science, School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan

[c] Division of Nephrology, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan

[d] Division of Cardiology, Department of Internal Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

*Corresponding author, e-mail: d0704@mail.cmuh.org.tw

Received 3 Feb 2010
Accepted 7 May 2010

ABSTRACT: Membranous glomerulonephritis (MGN) is one of the most common causes of idiopathic nephrotic syndrome in adults. *TLR4* gene polymorphisms have been reported to be associated with many inflammatory diseases. The objective of this study was to clarify the relationship between *TLR4* gene polymorphisms and the pathogenesis of MGN. We recruited a cohort of 134 biopsy-diagnosed MGN patients and 263 healthy subjects that served as controls. Genotyping of *TLR4* gene polymorphisms was performed using allele-specific polymerase chain reaction methods. We then analysed associations between *TLR4* gene polymorphisms and clinical manifestations and pathogenesis of MGN. There was statistically significant difference of *TLR4* gene rs10983755 A/G ($p < 0.001$) and rs1927914 A/G ($p < 0.05$) polymorphisms between controls and patients with MGN. The distributions of rs10759932 C/T and rs11536889 C/T polymorphisms were significantly different. A higher level of triglyceride was found in the non-GG group than in the GG group. The genotype of the non-AA group had a significantly higher ratio of proteinuria than that of the AA group. In addition, the distribution of haplotype frequencies of the *TLR4* gene in 4 genetic variants revealed no statistical difference between normal patients and controls. The results demonstrated that patients with MGN have a different genotype distribution of the *TLR4* gene from the normal controls. Our observations suggest that those polymorphisms contribute to the genetic background of MGN pathogenesis.

KEYWORDS: glomerulonephritis, inflammation, proteinuria, triglyceride

INTRODUCTION

Membranous glomerulonephritis (MGN) is a common primary or idiopathic nephropathy[1,2]. MGN also appears as a disease secondary to other conditions (approximately 25%), mainly infections, neoplasms, and systemic lupus erythematosus (SLE)[3]. Although inflammatory cells are not usually detected, an inflammatory process is evident of trapping of immune complexes. Therefore, inflammation is highly related to this disease[4]. MGN may be a cause of chronic kidney disease and a final result of end-stage renal disease (ESRD)[5]. Taiwan has the highest prevalence of ESRD in the world and MGN may be one of the causes[4,6,7]. The study of inflammatory factors associated with MGN is helpful in elucidating and preventing of ESRD[8].

MGN is an immune complexes mediated disease as evidenced by the presence of immunoglobulins and complement components in capillary walls (subepithelial), and the morphological and immunopathological similarities between the experimental MGN and immunological glomerular diseases[9]. However, the aetiology and origin of the antigens that cause MGN remain unclear. The deposits may come from circulating immune complexes, form in situ, or come from foreign antigens previously deposited there[10]. Although MGN is a multi-factorial disease, an inflammatory pathway might play an important role in the pathogenesis of MGN[4,9].

Toll-like receptors (TLRs), a key element of human innate immune response, up-regulate proinflammatory cytokines and co-stimulatory molecules as a first line host defence[11]. TLRs have been identified as key components of the pathogen-recognition process that mediates inflammatory responses[12]. TLR4 interacts with some ligands such as heat-shock proteins[13–15], hyaluronan, fibronectin, fibrinogen, heparan lung surfactant protein-A[16], and high mobility group box 1[17]. Polymorphisms of the *TLR4* gene have been reported to be associated with many inflammatory diseases and the formation of cancers such as Crohn's disease[18], ulcerative colitis[19], and cervical cancer[20]. However, there are few reports about TLR4 in MGN[21,22]. In this study, we investigated the association of *TLR4* gene variants in Taiwan MGN patients by comparing them with matched controls.

MATERIALS AND METHODS

Study population

A total of 134 patients with previously renal biopsy-approved MGN and 263 healthy subjects serving as controls were recruited in Taichung Veterans General Hospital, Taiwan. Patients with malignancy, chronic infection diseases (including infections with hepatitis B and C viruses), lupus nephritis, or drug-induced secondary MGN were excluded from the study. The general data (gender, body weight, systolic/diastolic pressure, and body height) and medical information (duration of follow-up, renal failure, and herbal use, etc.) of all the patients were reviewed. Patient characteristics included demographic variables, clinical and laboratory data in the disease courses, vascular events (cardiovascular disease and peripheral vascular events), and treatment regimens as well as their responses. The study was approved by the Institutional Review Board (No. C08159) of China Medical University Hospital in adherence with the Declaration of Helsinki and all participating individuals signed an informed consent.

The selection of treatment modality, either supportive or aggressive with immunosuppressants was decided by the treating physician. The supportive therapy usually included diuretics, angiotensin converting enzyme inhibitors and/or angiotensin II receptor blockers dependening on patient symptoms. The immunosuppressive therapies were any of the following regimens: (1) prednisolone 1 mg/kg/day alone, (2) a six-month course of corticosteroids alternated with chlorambucil at dose of 0.2 mg/kg per day every other month[4,23] or cyclophosphamide 1.5–2.0 mg/kg per day, (3) cyclosporine A (CyA, Neoral, Norvatis) 3–5 mg/kg per day with or without prednisolone.

Table 1 Genetic polymorphism sites of *TLR4* gene.

Polymorphism position	rs number	Assay ID
(5′UTR) A/G	rs10983755	C_31783994_10
(5′UTR) A/G	rs1927914	C_2704048_10
(5′UTR) C/T	rs10759932	C_31783996_10
(3′UTR) C/G	rs11536889	C_31784034_10

Responses and outcomes

The responses to therapy were defined as the follows: (1) no response, (2) partial remission: a proteinuria reduction of more than 50% or a final proteinuria between 0.2 to 2.0 g/day, and (3) complete remission: proteinuria less than 0.2 g/day. The 'progression of renal disease' was defined as a doubling of baseline serum creatinine (Cr) values or ESRD. ESRD was defined as patient requiring renal replacement therapy.

Genomic DNA extraction and determination of *TLR4* polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes (Genomic DNA kit, Roche). Genotypes of four SNPs (rs10983755, rs1927914, and rs10759932) representing 5′UTR A/G, 5′UTR A/G, and 5′UTR C/T polymorphism, respectively, and rs11536889 3′UTR C/G polymorphism in the *TLR4* gene (Table 1) were performed using the SNP genotyping assay (Applied Biosystems Inc. (ABI), Foster City). The primers and probes to detect for SNPs were from the ABI assay on demand kit. Reactions were carried out according to the manufacturer's protocol. Briefly, PCR was performed in the presence of 2× TaqMan Universal PCR Master Mix (ABI), assay mix (Applied Biosystems) and genomic DNA (15 ng). The probe for fluorescence signal detection was from the ABI Prism 7900 Real Time PCR System.

Statistical analyses

The Hardy-Weinberg equilibrium was tested for each marker using a χ^2-test. A χ^2 test or Fisher's exact test were used to determine statistically significant differences in allele/genotype frequencies. The haplotype combination of 4 polymorphisms in the *TLR4* gene was estimated using HAPLOVIEW version 4.1 based on an accelerated EM algorithm[24]. The differences in the distribution of the haplotype frequencies between the two groups were assessed using a χ^2-test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were obtained using logistic regressions to determine associations. All data were analysed with SPSS 15.0 and $p < 0.05$ was considered statistically significant.

Table 2 Genotypic and allelic frequencies of *TLR4* genetic polymorphisms in the patients with MGN and controls.

dbSNP ID	Patients with MGN	Control	OR (95% CI)	p value
rs10983755	n = 134	n = 265		
Genotype				
AA	8 (6.0)	12 (4.5)	2.03 (0.78–5.23)	0.0006
AG	75 (56.0)	98 (37.0)	2.33 (1.50–3.60)	
GG	51 (38.1)	155 (58.5)	Reference	
Allele freq.				
A	91 (34.0)	122 (23.0)	1.72 (1.24–2.38)	0.001
G	177 (66.8)	408 (77.0)	Reference	
rs1927914	n = 134	n = 265		
Genotype				
AA	44 (32.8)	121 (45.7)	Reference	0.045
AG	67 (50.0)	104 (39.2)	1.77 (1.12–2.81)	
GG	23 (17.2)	40 (15.1)	1.58 (0.85–2.93)	
Allele freq.				
A	155 (57.8)	346 (65.3)	Reference	0.040
G	113 (42.2)	184 (34.7)	1.37 (1.01–1.85)	
rs10759932	n = 134	n = 263		
Genotype				
CC	8 (6.0)	12 (4.6)	1.47 (0.57–3.75)	0.465
CT	56 (41.8)	97 (36.9)	1.27 (0.82–1.96)	
TT	70 (52.2)	154 (58.6)	Reference	
Allele freq.				
C	72 (26.9)	121 (23.0)	1.23 (0.88–1.72)	0.230
T	196 (73.1)	405 (77.0)	Reference	
rs11536889	n = 133	n = 261		
Genotype				
CC	6 (4.5)	11 (4.2)	1.16 (0.42–3.26)	0.560
CT	45 (33.8)	75 (28.7)	1.28 (0.81–2.01)	
TT	82 (61.7)	175 (67.0)	Reference	
Allele freq.				
C	57 (21.4)	97 (18.6)	1.19 (0.83–1.72)	0.341
T	209 (78.6)	425 (81.4)	Reference	

Table 3 Characteristics of clinical parameters for the GG and non-GG (rs10983755) of polymorphisms of the *TLR4* gene in patients with MGN.

Clinical parameter	*TLR4* (rs10983755)		
	GG (n = 51)	Non-GG (n = 83)	p-value
Male	26 (55.3)	47 (57.3)	0.83
Female	21 (44.7)	35 (42.7)	
Age (years)	52 ± 16	54 ± 18	0.56
Weight (kg)	63 ± 11	65 ± 11	0.89
Height (cm)	161 ± 8	160 ± 8	0.81
BMI (kg/m^2)	24.0 ± 3.5	25.3 ± 3.6	0.94
Systolic BP (mmHg)	136 ± 23	135 ± 19	0.62
Diastolic BP (mmHg)	82 ± 12	83 ± 12	0.51
Mean BP (mmHg)	100 ± 15	100 ± 13	0.32
Cholesterol (mg/dl)	357 ± 160	312 ± 100	0.09
Triglyceride (mg/dl)	187 ± 115	245 ± 172	0.02
Negative proteinuria	7 (14.9)	1 (1.2)	0.004
Positive proteinuria	40 (85.1)	81 (98.8)	

Data are expressed as n (%) or mean $\pm$ SD
BMI = body mass index, BP = blood pressure

RESULTS

There was a statistically significant difference of the *TLR4* gene rs10983755 A/G ($p < 0.001$) and rs1927914 A/G ($p < 0.05$) polymorphisms between controls and patients with MGN (Table 2). The frequency of the 'AA' genotype in patients with MGN (6.0%) was higher than in the control group (4.5%). By comparing with 'GG' genotype, the OR of 'AA' was 2.03 (95% CI = 0.78–5.23). The frequency of the 'AG' genotype in patients with MGN (56.0%) was also higher than in the control group (37.0%). By comparing with the 'GG' genotype, the OR of AG was 2.33 (95% CI = 1.50–3.60). The allelic frequency of 'A' in patients with MGN (34.0%) was higher than in the control (23.0%). The OR for the 'A' allele was 1.72 (95% CI = 1.24–2.38, $p = 0.001$).

For the distribution of rs1927914 A/G polymorphism, the 'GG' genotype was higher in the patient's group (17.2%) than in the control (15.1%) with an OR of 1.58 (95% CI = 0.85–2.93). The frequency of the the 'AG' genotype in patients with MGN (50.0%) was also higher than in the control group (39.2%) with an OR of 1.77 (95% CI = 1.12–2.81). The allelic frequency of 'G' in patients with MGN (42.2%) was higher than the control (34.7%) with an OR of 1.72 (95% CI = 1.01–1.85, $p < 0.05$).

The distribution of rs10759932 C/T and rs11536889 C/T polymorphisms were also shown. There was no significant difference of genotype frequency between these two groups.

The characteristics of clinical parameters in *TLR4* gene (rs10983755) A/G polymorphism revealed no difference except triglyceride ($p = 0.024$) between GG and non-GG genotype in MGN patients (Table 3). A higher level of triglyceride was found in the non-GG group than in the GG group (245 ± 172 and 187 ± 115 mg/dl, respectively). Non-GG genotype (98.8%) had a significantly ($p = 0.004$) higher percentage of proteinuria than the GG genotype (85.1%). Other parameters including renal failure, mortality, vascular event, CCr, C3, C4, immunoglobulins, results of biopsy (fibrosis or intima fibroplasia), disease progression and chronic kidney disease grade did not differ for the GG and non-GG genotype of rs10983755 in the *TLR4* gene.

There was also a statistically significant difference between the rs1927914 A/G polymorphism and the proteinuria parameter (Table 4). The genotype of the non-AA group (97.8%) had a significantly ($p = 0.011$) higher ratio of proteinuria than the AA group (85.0%). No statistically significant differences of rs10759932 C/T and rs11536889 C/T polymorphisms between TT and non-TT genotypes were found in the

Table 4 Characteristics of clinical parameters between AA and non AA (rs1927914) polymorphism of *TLR4* gene in patients with MGN.

Clinical parameters	*TLR4* (rs1927914)		
	AA ($n = 44$)	Non-AA ($n = 90$)	p-value
Male	27 (67.5)	46 (51.7)	0.09
Female	13 (32.5)	43 (48.3)	
Age (years)	53 ± 17	53 ± 17	0.99
Weight (kg)	64 ± 10	64 ± 11	0.29
Height (cm)	162 ± 7	160 ± 8	0.29
BMI (kg/m^2)	24.2 ± 3.2	25.1 ± 3.7	0.17
Systolic BP (mmHg)	139 ± 24	134 ± 18	0.29
Diastolic BP (mmHg)	82 ± 12	83 ± 12	0.996
Mean BP (mmHg)	101 ± 15	100 ± 13	0.39
Cholesterol (mg/dl)	310 ± 130	336 ± 125	0.94
Triglyceride (mg/dl)	200 ± 136	234 ± 163	0.30
Negative proteinuria	6 (15.0)	2 (2.2)	0.01
Positive proteinuria	34 (85.0)	87 (97.8)	

Table 5 Distribution of TLR4 haplotype frequencies in the patients with MGN and controls.

Haplotype[a]	Patient with MGN[b] ($n = 138$)	Control ($n = 265$)	OR (95% CI)	p-value
G-A-T-G	32.0%	37.9%	0.77 (0.57–1.06)	0.10
A-G-C-G	26.0%	22.5%	1.22 (0.87–1.72)	0.27
G-A-T-C	20.3%	23.9%	0.80 (0.56–1.15)	0.24
G-G-T-G	13.3%	14.8%	0.89 (0.58–1.36)	0.57

[a] Order of single nucleotide polymorphisms comprising the *TLR4* haplotypes: rs10983755, rs1927914, rs10759932, and rs11536889.

[b] Percentages may not add to 100% because of the presence of rare haplotypes ($< 5\%$) not presented here.

clinical parameters (data not shown).

In addition, compared with the haplotype frequencies between patient and control groups, the distribution of haplotype frequencies of *TLR4* gene in 4 genetic variants revealed no statistical difference between MGN patients and controls (Table 5).

DISCUSSION

MGN is a multiple factorial disease with immunologic expressions that may arise in genetically susceptible individuals[4,9]. However, little information is available for the polymorphic gene sequences of inflammatory genes known to be involved in pathogenesis of MGN[25–27]. In this study, we focused on the variants of *TLR4* which had previously been investigated for diabetic neuropathy[28], severe virus infections[29], cervical cancer susceptibility[30], and chronic allograft nephropathy[31]. According to our data, we found a statistically significant association between MGN and the *TLR4* gene, rs10983755 A/G, and rs1927914 A/G polymorphisms.

The genetic basis of MGN is not fully understood. MGN patients show significantly lower renal expression of tumour necrosis factor (TNF-α) than patients with minimal change disease, diabetes nephropathy, IgA nephropathy, or other diseases[32]. A recent report shows that the *TNF*-α gene G-308A polymorphism is a risk factor for the development of MGN[33]. Other reports show no relationship between severity or progression of the disease and any of the tested single genotypes [HLA-DR3, *TNF*-α gene G-308A, angiotensin-converting enzyme insertion/deletion (ACE I/D), angiotensin II receptor 1 (AT1R 1166A/C), angiotensinogen (AGT M235T), and NOS (ecNOS4b/a)][34,35], perhaps due to the smaller number of patients and short observation periods.

The renal function changes and the course of MGN are more strongly correlated with the degree of tubulointerstitial damage than with the extent of the glomerular lesion[36]. However, the pathogenesis of the interstitial inflammation and fibrosis is unclear. The most frequent presentation is proteinuria in the nephrotic range, with or without the other findings of the complete nephrotic syndrome. A variable percentage of cases present as asymptomatic proteinuria, with microscopic haematuria in most of the patients, but macrohaematuria is rare. Exceptionally it can appear with isolated haematuria. The renal function can be slightly altered at the time of the diagnosis in many cases, but renal failure is unusual at presentation. Furthermore, MGN may be influenced by risk factors such as microalbuminuria or hypoglycaemia. Because these risk factors were found to be distributed differently in the groups (*TLR4* gene rs10983755 A/G and rs1927914 A/G) in this study, it seems that the effects of these *TLR4* genotypes on MGN are related to these risk factors in these patients.

The amino acid polymorphisms determine the differences in the structure and thus the pattern recognition of the TLR4 receptor[37]. One might suggest that an abnormality in the TLR regulation might increase the susceptibility for diseases because of a reduced defence against invading pathogens[38]. Diagnosis of MGN can only be made after excluding secondary causes. Therefore, for proper diagnosis, investigations based on history, serology, and histology are equally important as a deficiency could arise at any level. MGN has a histological appearance, which could be due to factors such as heavy metal exposure, hepatitis B or C[39,40], Epstein-Barr virus[41], parasites, *Heli-*

cobacter pylori infection[42], SLE, or even neoplastic conditions[43]. To date, no data are available concerning the function and expression of TLR4 in MGN. The pattern recognition receptor TLR4 is known to activate the proinflammatory transcription factor (NF)-κB and subsequent gene expression of NF-κB-regulated genes such as cytokines and adhesion molecules. This indicates that a TLR-4 polymorphism that prevents ligand binding and subsequent cellular signalling would result in lower NF-κB activation and subsequent NF-κB-dependent proinflammatory gene expression[44]. Hence, further studies are required to show whether the polymorphisms are associated with a reduced activation of NF-κB, cytokine expression, and expression of adhesion molecules in MGN patients.

In the present study, clinical features of 129 patients regarding vascular events (including unstable angina, coronary artery disease, ischaemic heart disease, renal artery or vein thrombosis, deep vein thrombosis and cranial vascular events) were available for review and data analysis. We observed all MGN patients had high levels of cholesterol (reference: 150–240 mg/dl) and 46% (59/129) MGN patients with vascular events problem (38% (18/47) with GG genotype at rs10983755; 50% (41/82) with Non-GG genotype at rs10983755). Comparing intergroup genotype (GG and Non-GG) gave statistically significant differences in triglycerides level for rs10983755 SNP ($p = 0.024$) in MGN patients. Data indicated triglycerides as 'at-risk' for vascular event development in MGN patients with Non-GG genotype. On the other hand, patients with MGN presented varied signs and symptoms, most commonly haematuria (75%), proteinuria (50%), and oedema (38%), consistent with other publications. Patients may have hypoalbuminaemia but most have hypercholesterolemia[45]. The value of proteinuria is as a marker for the MGN patients' responses to therapy.

The interpretation of our study results is limited because the patients were recruited from just one centre in Taiwan. Our results strongly suggest a significant role of *TLR4* gene polymorphisms in the risk of developing MGN of Taiwan. To the best of our knowledge, this is the first report on *TLR4* gene polymorphisms in MGN patients. However, the identification of *TLR4* as genetic risk factors for MGN susceptibility in Taiwan may be further evaluated as prognostic markers for predictive clinical testing in MGN worldwide, especially in ethnically disparate populations. Additionally, the study lacks functional genetics, the functional activity of the two identified polymorphisms in peripheral blood lymphocytes or the expression of TLR-4 in renal biopsies of MGN patients and other glomerulopathies should be further studied. More recently, Hwang et al showed the importance of TLR4 in the pathogenesis of acute rejection in kidney transplantation, although there was no difference in transcriptional activity between wild-type and variant promoter of *TLR4*[46]. This means that there is no functional significance in this promoter SNPs of *TLR4*. The DNA variant responsible for the difference in the genetic background of MGN pathogenesis remains to be identified.

In summary, our study firstly demonstrated the different genotype distribution between normal controls and patients with MGN of the *TLR4* gene. The data show that *TLR4* gene may be associated with a disease clinical cause of MGN especially with respect to the clinical parameters of proteinuria and serum triglyceride levels. The *TLR4* gene is one of a number of important inflammatory related genes. Our observations suggest that these polymorphisms contribute to the genetic background of MGN pathogenesis.

Acknowledgements: This study was supported by grants from National Science Council of Taiwan (NSC 98-2314-B-039-023-MY3 and NSC 97-2320-B-039-022-MY3) and China Medical University (CMU97-CMC-003 and DMR-97-125), Taichung, Taiwan. The authors thank Miss Chi-Hsiang Wei for manuscript preparation.

REFERENCES

1. Appel AS, Appel GB (2009) An update on the use of mycophenolate mofetil in lupus nephritis and other primary glomerular diseases. *Nat Clin Pract Nephrol* **5**, 132–42.
2. Mok CC (2009) Membranous nephropathy in systemic lupus erythematosus: a therapeutic enigma. *Nat Rev Nephrol* **5**, 212–20.
3. Glassock RJ (1992) Secondary membranous glomerulonephritis. *Nephrol Dial Transplant* **7 Suppl 1**, 64–71.
4. Ponticelli C (2007) Membranous nephropathy. *J Nephrol* **20**, 268–87.
5. Philibert D, Cattran D (2008) Remission of proteinuria in primary glomerulonephritis: we know the goal but do we know the price? *Nat Clin Pract Nephrol* **4**, 550–9.
6. Chen KH, Chang CT, Hung CC (2006) Glomerulonephritis associated with chronic inflammatory demyelinating polyneuropathy. *Ren Fail* **28**, 255–9.
7. Yen TH, Huang JY, Chen CY (2003) Unexpected IgA nephropathy during the treatment of a young woman with idiopathic dermatomyositis: case report and review of the literature. *J Nephrol* **16**, 148–53.
8. Lo WY, Chen SY, Wang HJ, Shih HC, Chen CH, Tsai CH, et al (2010) Association between genetic polymorphisms of the NPHS1 gene and membranous

glomerulonephritis in the Taiwanese population. *Clin Chim Acta* **411**, 714–8.
9. Couser WG, Nangaku M (2006) Cellular and molecular biology of membranous nephropathy. *J Nephrol* **19**, 699–705.
10. Ronco P, Debiec H (2006) New insights into the pathogenesis of membranous glomerulonephritis. *Curr Opin Nephrol Hypertens* **15**, 258–63.
11. Lee CH, Wu CL, Shiau AL (2010) Toll-like receptor 4 signaling promotes tumor growth. *J Immunother* **33**, 73–82.
12. Medzhitov R, Janeway C, Jr (2000) The Toll receptor family and microbial recognition. *Trends Microbiol* **8**, 452–6.
13. Lin FY, Chen YH, Chen YL, Wu TC, Li CY, Chen JW, et al (2007) Ginkgo biloba extract inhibits endotoxin-induced human aortic smooth muscle cell proliferation via suppression of toll-like receptor 4 expression and NADPH oxidase activation. *J Agr Food Chem* **55**, 1977–84.
14. Lin FY, Chen YH, Tasi JS, Chen JW, Yang TL, Wang HJ, et al (2006) Endotoxin induces toll-like receptor 4 expression in vascular smooth muscle cells via NADPH oxidase activation and mitogen-activated protein kinase signaling pathways. *Arterioscler Thromb Vasc Biol* **26**, 2630–7.
15. Lin FY, Chen YH, Lin YW, Tsai JS, Chen JW, Wang HJ, et al (2006) The role of human antigen R, an RNA-binding protein, in mediating the stabilization of toll-like receptor 4 mRNA induced by endotoxin: a novel mechanism involved in vascular inflammation. *Arterioscler Thromb Vasc Biol* **26**, 2622–9.
16. Beg AA (2002) Endogenous ligands of Toll-like receptors: implications for regulating inflammatory and immune responses. *Trends Immunol* **23**, 509–12.
17. Liu PL, Tsai JR, Hwang JJ, Chou SH, Cheng YJ, Lin FY, et al (2009) HMGB1-Mediated MMP-9 Expression in Non-Small Cell Lung Cancer Contributes to Tumor Cell Invasiveness. *Am J Respir Cell Mol Biol*.
18. Zouiten-Mekki L, Kharrat M, Karoui S, Serghimi M, Fekih M, Matri S, et al (2009) Tolllike receptor 4 (TLR4) polymorphisms in Tunisian patients with Crohn's disease: genotype-phenotype correlation. *BMC Gastroenterol* **9**, 62.
19. Rigoli L, Romano C, Caruso RA, Lo Presti MA, Di Bella C, Procopio V, et al (2008) Clinical significance of NOD2/CARD15 and Toll-like receptor 4 gene single nucleotide polymorphisms in inflammatory bowel disease. *World J Gastroenterol* **14**, 4454–61.
20. Cristofaro P, Opal SM (2006) Role of Toll-like receptors in infection and immunity: clinical implications. *Drugs* **66**, 15–29.
21. Brown HJ, Lock HR, Wolfs TG, Buurman WA, Sacks SH, Robson MG (2007) Toll-like receptor 4 ligation on intrinsic renal cells contributes to the induction of antibody-mediated glomerulonephritis via CXCL1 and CXCL2. *J Am Soc Nephrol* **18**, 1732–9.
22. Robson MG (2009) Toll-like receptors and renal disease. *Nephron Exp Nephrol* **113**, e1–7.
23. Passerini P, Ponticelli C (2003) Corticosteroids, cyclophosphamide, and chlorambucil therapy of membranous nephropathy. *Semin Nephrol* **23**, 355–61.
24. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263–5.
25. Baek JI, Choi SJ, Park SH, Choi JY, Kim CD, Kim YL, et al (2009) Identification of novel variants in the COL4A4 gene in Korean patients with thin basement membrane nephropathy. *Indian J Med Res* **129**, 525–33.
26. Chen CH, Shu KH, Wen MC, Chen KJ, Cheng CH, Lian JD, et al (2008) Impact of plasminogen activator inhibitor-1 gene polymorphisms on primary membranous nephropathy. *Nephrol Dial Transplant* **23**, 3166–73.
27. Rana K, Wang YY, Buzza M, Tonna S, Zhang KW, Lin T, et al (2005) The genetics of thin basement membrane nephropathy. *Semin Nephrol* **25**, 163–70.
28. Rudofsky G, Jr, Reismann P, Witte S, Humpert PM, Isermann B, Chavakis T, et al (2004) Asp299Gly and Thr399Ile genotypes of the TLR4 gene are associated with a reduced prevalence of diabetic neuropathy in patients with type 2 diabetes. *Diabetes Care* **27**, 179–83.
29. Tulic MK, Hurrelbrink RJ, Prele CM, Laing IA, Upham JW, Le Souef P, et al (2007) TLR4 polymorphisms mediate impaired responses to respiratory syncytial virus and lipopolysaccharide. *J Immunol* **179**, 132–40.
30. Pandey S, Mittal RD, Srivastava M, Srivastava K, Singh S, Srivastava S, et al (2009) Impact of Toll-like receptors [TLR] 2 (-196 to -174 del) and TLR 4 (Asp299Gly, Thr399Ile) in cervical cancer susceptibility in North Indian women. *Gynecol Oncol* **114**, 501–5.
31. Mutlubas F, Mir S, Berdeli A, Ozkayin N, Sozeri B (2009) Association between Toll-like receptors 4 and 2 gene polymorphisms with chronic allograft nephropathy in Turkish children. *Transplant Proc* **41**, 1589–93.
32. Wu TH, Tsai CY, Yang WC (1998) Excessive expression of the tumor necrosis factor-alpha gene in the kidneys of patients with membranous glomerulonephritis. *Zhonghua Yi Xue Za Zhi (Taipei)* **61**, 524–30.
33. Bantis C, Heering PJ, Aker S, Siekierka M, Kuhr N, Grabensee B, et al (2006) Tumor necrosis factor-alpha gene G-308A polymorphism is a risk factor for the development of membranous glomerulonephritis. *Am J Nephrol* **26**, 12–5.
34. Stratta P, Bermond F, Guarrera S, Canavese C, Carturan S, Dall'Omo A, et al (2004) Interaction between gene polymorphisms of nitric oxide synthase and renin-angiotensin system in the progression of membranous glomerulonephritis. *Nephrol Dial Transplant* **19**, 587–95.
35. Cattran DC (2001) Idiopathic membranous glomerulonephritis. *Kidney Int* **59**, 1983–94.
36. Wehrmann M, Bohle A, Bogenschutz O, Eissele R,

Freislederer A, Ohlschlegel C, et al (1989) Long-term prognosis of chronic idiopathic membranous glomerulonephritis. An analysis of 334 cases with particular regard to tubulo-interstitial changes. *Clin Nephrol* **31**, 67–76.

37. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, et al (2000) TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* **25**, 187–91.
38. Schwartz DA (2002) TLR4 and LPS hyporesponsiveness in humans. *Int J Hyg Environ Health* **205**, 221–7.
39. Tang S, Lai FM, Lui YH, Tang CS, Kung NN, Ho YW, et al (2005) Lamivudine in hepatitis B-associated membranous nephropathy. *Kidney Int* **68**, 1750–8.
40. Ozdemir BH, Ozdemir FN, Sezer S, Colak T, Haberal M (2006) De novo glomerulonephritis in renal allografts with hepatitis C virus infection. *Transplant Proc* **38**, 492–5.
41. Araya CE, Gonzalez-Peralta RP, Skoda-Smith S, Dharnidharka VR (2006) Systemic Epstein-Barr virus infection associated with membranous nephropathy in children. *Clin Nephrol* **65**, 160–4.
42. Nagashima R, Maeda K, Yuda F, Kudo K, Saitoh M, Takahashi T (1997) *Helicobacter pylori* antigen in the glomeruli of patients with membranous nephropathy. *Virchows Arch* **431**, 235–9.
43. Dash SC, Al-Muhanna FA (2005) Unresolved issues and current concepts in management of primary glomerulonephritis. *Ann Saudi Med* **25**, 329–34.
44. Mezzano SA, Barria M, Droguett MA, Burgos ME, Ardiles LG, Flores C, et al (2001) Tubular NF-κB and AP-1 activation in human proteinuric renal disease. *Kidney Int* **60**, 1366–77.
45. Chen A, Frank R, Vento S, Crosby V, Chandra M, Gauthier B, et al (2007) Idiopathic membranous nephropathy in pediatric patients: presentation, response to therapy, and long-term outcome. *BMC Nephrol* **8**, 11.
46. Hwang YH, Ro H, Choi I, Kim H, Oh KH, Hwang JI, et al (2009) Impact of polymorphisms of TLR4/CD14 and TLR3 on acute rejection in kidney transplantation. *Transplantation* **88**, 699–705.