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Keywords: membranous glomerulonephritis (MGN); signal transducer and activator of transcription 4 (STAT4); Single nucleotide polymorphisms (SNPs); Haplotype.

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Abstract: Background: Membranous glomerulonephritis (MGN) is one of common causes of idiopathic nephrotic syndrome in adults, and 25% of MGN patients proceed to end-stage renal disease. STAT4 gene polymorphisms have been reported to be associated with many inflammatory diseases. The objective of this study was to clarify the relationship between STAT4 gene polymorphisms and the pathogenesis of MGN.
Methods: We investigated the association of three STAT4 gene polymorphisms (rs3024912, rs3024908, and rs3024877) with the susceptibility to MGN in 403 Taiwanese populations (138 MGN patients and 265 controls).
Results: The results indicated that the statistically significant difference in genotype frequency distribution was found at rs3024908 SNP in MGN patients and control groups ($p = 0.014$). In addition, the individuals with the GG genotype at rs3024912 SNP may have a higher risk in kidney failure of MGN patients (adjusted odds ratio [OR] = 3.255; 95% confidence interval [CI] = 1.155-9.176, $p = 0.026$).
Conclusions: Our data provide a new information that the STAT4 (rs3024912 and rs3024908) polymorphisms may be the underlying cause of MGN, and these polymorphisms revealed by this study warrant further investigation.

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Article Title: **Association of STAT4 Polymorphisms with Susceptibility to Primary Membranous Glomerulonephritis and Renal Failure**.

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Dear Prof. Lam Jun 03, 2011

Thank you so much for your letter and referee(s)' comments on our manuscript (code CCA-D-10-01239). Enclosed please find a copy of the revised manuscript and the point to point reply to the comments and questions raised by reviewers.

If you have any further comments or questions, please feel free to contact me at the following numbers: Phone: +886-4-22052121 ext 2033; email: chenshihy@yahoo.com.tw; chenshihy@mail.cmu.edu.tw

Best regards

Sincerely,

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Reviewer Comments 1:

Please clarify how the controls were obtained, and what workup was performed to exclude occult renal disease.

Response:

We would like to thank the reviewer for these comments. For explaining the point which reviewer mention about, we had some description in the section of Materials and Methods (please see p. 6, line 5-8).

Reviewer Comments 2:

Is the distribution of polymorphism similar to other population / reported series?

Response:

We appreciate this helpful comment. To our knowledge, this is the first report on *STAT4* polymorphisms in MGN patients. Currently, compared with other studies using Asian individual groups, the similarly distribution of these polymorphisms were obtained. However, we have the biggest sample size.

Reviewer Comments 3:

Please provide more information on baseline clinical and pathological information, including baseline proteinuria, renal function, blood pressure, histological scarring, and treatment.

Response:

For this helpful comment, as the reviewer has suggested, we add the

information in Table 4 (please see p. 29) and the description in the section of Materials and Methods (please see p. 7, line 2-8), the section of Result (please see p. 12, line 7-17) and the section of Discussion (please see p. 16, line 11-16).

Reviewer Comments 4:

What was the average duration of observation? Please provide Kaplan Meire curve for different genotypes.

Response:

For this helpful comment, as the reviewer has suggested, we add the information in Figure 2 (please see p. 25) and the description in the section of Materials and Methods (please see p. 9, line 14-16) and the section of Result (please see p. 13, line 14-19 and p. 14, line 1-7).

Reviewer Comments 5:

Is there any information of rate of GFR decline? Progress to renal failure is a valid end point but would bias towards more severe / rapidly progressive cases.

Response:

We appreciate this helpful comment. We add table 5 and using pathological features for data analysis. We also add the description in the section of Materials and Methods (please see p. 7, line 9-17) and the section of Result (please see p. 12, line 2-12).

ABSTRACT

Background: Membranous glomerulonephritis (MGN) is one of common causes of idiopathic nephrotic syndrome in adults, and 25% of MGN patients proceed to end-stage renal disease. *STAT4* gene polymorphisms have been reported to be associated with many inflammatory diseases. The objective of this study was to clarify the relationship between *STAT4* gene polymorphisms and the pathogenesis of MGN.

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Association of STAT4 Polymorphisms with Susceptibility to Primary Membranous Glomerulonephritis and Renal Failure

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Key words:

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1. Introduction

Membranous glomerulonephritis (MGN), the common cause of nephrotic syndrome, accounts for approximately 40% of adult cases [1]. It is characterized by basement membrane thickening and subepithelial immune deposits without cellular proliferation or infiltration [2]. Previous study suggested MGN as causing chronic kidney disease (CKD) and as a final result of end-stage renal disease (ESRD) [3]. Therapies for MGN that include the use of immunosuppressive drugs and nonspecific antiproteinuric measures have led to disappointing results and prompted increased interest in the discovery of new therapeutic targets [4]. Taiwan has the highest prevalence of ESRD worldwide and MGN may be one cause [5-7]. Study of inflammatory factors associated with MGN is helpful for the elucidating and preventing of ESRD.

The signal transducer and activator of transcription 4 (*STAT4*) gene, located on chromosome 2q32.2-32.3, encodes a transcription factor which plays an essential role in the development of inflammation of various immune-mediated diseases [8].

Likewise, *STAT4* plays a crucial role in regulation of the immune response by transmitting signals activated in response to several cytokines, including type 1 IFN, IL-12, and IL-23 [9]. *STAT4* is necessary for IL-12 induced differentiation of naïve CD4+ T cells into Th1 cells, and activated Th1 cells drive chronic inflammation, by secreting high levels of pro-inflammatory cytokines like IFN-γ and TNF-α [10]. In addition, recent studies demonstrated that *STAT4* is also responsible for the expansion of Th17 cells activated by IL-23 [11], which promotes chronic inflammation in adaptive and innate immunity and contributes to the development of a variety of autoimmune diseases [9, 12-14]. Moreover, it was shown that *STAT4* haplotype characterized by the rs7574865 polymorphism was reported to be significantly associated with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and other autoimmune diseases such as Sjögren's syndrome, type I diabetes, and systemic sclerosis [15-19], but no genetic study regarding the relationship of such polymorphisms with MGN disease.

The present study aimed to identify genetic polymorphisms in potential candidate genes for MGN, and we therefore investigated the association of *STAT4* gene polymorphisms with MGN in a Taiwanese population. Our findings are expected to help us understand the role of *STAT4* gene polymorphisms in MGN disease and its progression; this knowledge can point us toward possible management strategies for

this common nephropathy.

2. Materials and Methods

2.1. Study Population

A gender-age-matched control group composed of 265 non-diabetic, non-nephropathic, normotensive healthy unrelated control subjects, whom identified through health examination at Taichung Veterans General Hospital in Taiwan, was enrolled. We also recruited 138 patients with the previously renal biopsy-approved membranous glomerulonephritis (MGN) in the same Hospital during 1982-2008. The 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, real failure, and herbal use, etc.) of all the patients were reviewed. Patient characteristics includes: 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. All participants signed informed consent. The study was approved by the institutional review board of the hospital (VGHTC IRB No. C08159).

2.2. Response and Outcomes

The responses to therapy were defined as the follows: (a) no response, (b) partial remission: a proteinuria reduction more than 50% or a final proteinuria between 0.2 to 2.0 g/day, and (c) 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 in ESRD. ESRD was defined as patient requiring renal replacement therapy.

2.3. Renal Biopsy Review

Histological staging was based on histological lesion, including glomerular lesion [20], tubulointerstitial lesion, focal glomerulosclerosis [21], and fibrointimal lesion. Biopsy specimens were reviewed by a nephropathologist, who was unaware of patients' clinical history, renal function and *STAT4* gene SNPs (rs3024912, rs3024908, and rs3024877). Semiquantitative scoring system used a scale of 0 (absent), 1 (mild: <25%) and 2 (moderate to severe: >25%) for the assessment of tubulointerstitial change and glomerular sclerosis/obsolescence under light microscopy. Staging of the disease was determined according to finding under electron microscopy [22, 23].

2.4. SNP selection

STAT4 SNPs genotypes informations were downloaded in December 2008 from

the HapMap CHB + JPT population. HapMap genotypes were analyzed within Haploview and Tag SNPs were selected using the Tagger function by applying the following additional criteria: (i) a threshold minor allele frequency (MAF) in the HapMap CHB + JPT population of 0.05 for "tag SNPs"; and (ii) probe/primers that pass the qualification as recommended by the manufacturer (Applied Biosystems), to ensure a high genotyping success rate. Three polymorphisms met the criteria and were selected, including SNP rs3024912 (G/T) in 3'UTR, SNP rs3024908 (A/G) in 3'UTR, and SNP rs3024877 (A/G) in intron 15 of *STAT4* gene (Figure 1).

2.5. Genomic DNA Extraction and Genotyping of STAT4 Gene Genetic Polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Genomic DNA kit; Roche, USA). Genotypes of three SNPs (rs3024912, rs3024908, and rs3024877) at chromosome positions 2:191893087 (3'UTR), 2:191894141 (3'UTR), and 52198412 (intron 15) in *STAT4* gene (Figure 1) were performed using the Taqman SNP genotyping assay (ABI: Applied Biosystems Inc. Foster City, CA, USA). The primers and probes of SNPs were from the ABI assay on demand (AOD) kit. Reactions were carried out according to the manufacturer's protocol. Briefly, polymerase chain reaction (PCR) was performed in

the presence of 2× TaqMan® Universal PCR Master Mix (ABI, Foster City, CA, USA), assay mix (Assay ID C_15984893_10, C_15984883_10, and C_15984786_10, Applied Biosystems, USA) and genomic DNA (15 ng). After initial denaturation for 10 min at 95°C, 40 cycles were run, each consisting of denaturation (95°C for 15 s), and annealing (60°C for 60 s). The probe fluorescence signal detection was performed using the ABI Prism 7900 Real Time PCR System.

2.6. Statistical Analysis

Chi-square test or Fisher's exact tests determined statistically significant differences in allele/genotype frequencies between case and control groups. Allelic frequencies were expressed as percentage of the total alleles. Hardy–Weinberg equilibrium was tested for each marker using χ^2-test. Odds ratios [ORs] and 95% confidence intervals (95% CIs) were derived by logistic regressions to correlate *STAT4* alleles/genotypes/haplotypes with MGN susceptibility. The Kaplan-Meier method was used to estimate cumulative survival. Differences in survival were analyzed with the log-rank test. All data were analyzed with SPSS Version 15.0 software (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

3. Results

3.1. Genotypic and allelic frequencies of STAT4 genetic polymorphisms in MGN patients and controls

Table 1 plots allelic and genotypic frequencies of rs3024912, rs3024908, and rs3024877, genotype distributions in Hardy-Weinberg equilibrium. We observed the A allele as the major one at rs3024908 polymorphism both in MGN patients (85%; 233/274) and controls (85%; 453/530). There was no statistically significant difference in allelic frequencies distributions at rs3024912 and rs3024877 SNP. When we compared the genotype frequencies between MGN patients and control groups, a statistically significant difference in genotype frequency distributions was noted for rs3024908 SNP in MGN patients and controls (p = 0.014). Our data indicated that individuals with the AA genotype at rs3024908 SNP may have a higher risk of developing MGN.

3.2. Distribution of SATA4 haplotype frequencies in MGN patients and controls

The Haplotype frequencies were estimated using the rs3024912, rs3024908, and rs3024877 SNPs (Table 2). Five haplotypes of *STAT4* were present in our study population. Ht 1 (G-A-A) and Ht 2 (T-A-G) were the common haplotypes, both in

MGN patients (28.3% and 25.1%, respectively) and control groups (33.7% and 24.1%, respectively). Comparison of the haplotype frequencies between case and control groups indicated that the Ht 1 and Ht 5 haplotypes appeared to be the "protective" haplotypes as compared with others (OR: 0.77, 95% CI: 0.56–1.05, $p = 0.114$ at Ht 1 haplotype; OR: 0.59, 95% CI: 0.31–1.31, $p = 0.119$ at Ht 5 haplotype). However, the Ht 3 haplotype appeared to be an "at-risk" haplotype for MGN progression (OR: 1.34, 95% CI: 0.89–2.00; $p = 0.163$), although the difference was not statistically significant (Table 2).

3.3. Association between STAT4 genotypes and MGN patients with/without kidney failure

The Logistic Regression test was used for association analysis between *STAT4* genotypes and MGN patients with/without kidney failure. Renal failure was observed in 21 patients during the follow-up period with an incidence of 15.22 % (21/138) (Table 3). Median and mean renal survival durations were 8.0 and 5.7 years, respectively. In addition, 12 patients died during the follow-up period, and the mortality in our population was 8.70 % (12/138). Median and mean survival durations were 9.6 and 12.9 years, respectively. The longest follow-up period for patients who died before the end-point of this study was 17.4 years; for surviving patients, the longest period was 22.7 years (data not shown). Comparison of the genotype

frequencies between MGN patients and control groups, a statistically significant difference was noted for rs3024912 SNP in MGN patients and controls (OR: 2.947, 95% CI: 1.074-8.092, $p = 0.041$). Yet, we also observed a strong correlation in MGN patients and controls after adjusting gender and follow-up period effect (OR: 3.255, 95% CI: 1.155-9.176, $p = 0.026$). Our data indicated that individuals with the GG genotype at rs3024912 SNP may have a higher risk in kidney failure of MGN patients (Table 3).

3.4. Association between STAT4 major Ht 1 haplotype and clinical features in MGN patients

A comparison of the clinical features of MGN patients with/without the major Ht 1 haplotype is shown in Table 4. There were no differences in age of onset, duration of follow up, body mass index (BMI), mean blood pressure (MBP), and incidence of hematuria or proteinuria. After a mean 12.9 ± 6.2 years follow-up period, we observed the creatinine clearance (CCr) levels in the last laboratory test to be 64.2 ± 43.55 ml/min in MGN patients with Ht 1 haplotype and 47.07 ± 34.9 ml/min in those with the non Ht 1 haplotype ($p = 0.017$). However, the initial laboratory tests revealed no differences in the baseline creatinine levels (Cr) and daily urinary protein excretion (DUP).

3.5. Association between STAT4 major Ht 1 haplotype and pathological features in MGN patients

We also analyzed the relationship between the *STAT4* major Ht 1 haplotype and the pathological features of MGN. The scoring for MGN requires electron microscopy images of the glomeruli; however, only 107 biopsy specimens were available for review and scoring by the pathologist. There were 27 glomeruli (25.2%) at stage I, 55 (51.4%) at stage II, 18 (16.8%) at stage III, 5 (4.7%) at stage IV, and 2 (1.9%) at stage V. As shown in Table 5, there were no differences in the results for histological examination, percentage of global sclerosis, tubulointerstitial fibrosis and the fibrointimal atherosclerosis score between with/without Ht 1 haplotype of MGN patients.

3.6. Association between STAT4 major Ht 1 haplotype and survival status in MGN patients

The log-rank test was used for survival analysis of MGN patients with/without the A-G haplotype of the *STAT4* gene. As shown in Fig. 2A, renal failure was observed in 21 patients during the follow-up period with an incidence of 15.79 % (21/133). Median and mean renal survival durations were 8.0 and 5.7 years,

respectively. In addition, 12 patients died during the follow-up period, and the mortality in our population was 9.02 % (12/133). Median and mean survival durations were 9.6 and 12.9 years, respectively. The longest follow-up period for patients who died before the end-point of this study was 17.4 years; for surviving patients, the longest period was 22.7 years (Fig. 2B). The Kaplan-Meier curves for renal and patient survival showed that there was no statistically significant difference between the Ht 1 and non Ht 1 haplotypes of the *STAT4* gene in MGN patients (Fig. 2).

4. Discussion

Currently, MGN is considered to be an infectious disease with immunologic expression that occurs in genetically susceptible individuals [24, 25]. Polymorphic gene sequences of cytokines known to be involved in the pathogenesis of MGN are potential markers of disease susceptibility. Previous studies relatied the incidence of MGN and several polymorphisms, including TNF-α gene G-308A, ACE insertion or deletion (ACE I/D), angiotensin II receptor 1 (AT1R 1166A/C), angiotensinogen (AGT M235T), and NOS (ecNOS4b/a) [26-28]. In the present investigation we observed a correlation between the risk genotype of *STAT4* and a higher frequency of kidney failure among MGN patients with the risk genotype, which suggests that polymorphisms in *STAT4* contribute to the underlying autoimmune process in MGN.

This effect could be due to the many different effects of STAT4 in the immune system. Besides its role in type I IFN signaling, STAT4 also transmits signals from, e.g. IL-12 and IL-23, and is thus responsible for the IL-12-dependent activation of natural killer (NK) cells and production of IFN- γ, as well as for polarization of naive CD4+ T-cells to IFN-γ producing Th1 effector cells [29].

STAT4, which plays a pivotal role in Th1 immune responses, enhances IFN-γ transcription in response to the interaction of IL-12 with the IL-12 receptor [30, 31]. Yet, STAT4-deficient mice lack many IL-12-stimulated responses, including the induction of IFN-γ secretion and the differentiation of Th1 cells [32, 33]. These mice are generally resistant to autoimmune diseases such as proteoglycan-induced arthritis, experimental autoimmune encephalomyelitis, and diabetes [9]. Collectively, these findings strongly indicate that a deficiency of STAT4 expression is directly associated with impaired Th1 responses and associated immune diseases. However, the molecular mechanisms for transcriptional or post-transcriptional regulation of STAT4 expression have not yet been elucidated.

In this study, we focused on the variants of the *STAT4* gene (rs3024912, rs3024908, and rs3024877) that had previously been investigated for systemic lupus erythematosus (SLE) and cardiovascular disease events [34, 35]. We found a statistically significant association between MGN and the rs3024908 polymorphism.

The AA genotype frequency at rs3024908 was higher in MGN than in the control participants (Table 1). Our results also indicated that the Ht1 haplotype of the *STAT4* gene was estimated to be present in approximately 28.3% of MGN patients. Compared with control group, the Ht1 haplotype seems appeared to be a susceptibility factor for preventing MGN in our Taiwanese cohort, although the difference was not statistically significant (Table 2).

The treatment strategies for patients with MGN have been a subject of much controversy. In our series, most the patients were treated with ACE inhibitors (ACEIs) or angiotensin receptor blockers (ARBs). Despite the similar mode of treatment given to our patients, 15.22 % (21/138) kidney failure cases were observed in MGN subgroup. As shown in Table 3, after considering the gender and follow-up period effect, individuals with the GG genotype at rs3024912 SNP may have a higher risk in kidney failure of MGN patients. We observed that the latest CCr level in MGN patients with non Ht 1 haplotype was significantly lower than in patients with Ht 1 haplotype (47.07 ± 34.9 ml/min and 64.2 ± 43.55 ml/min, respectively) (p = 0.017). Despite the similar mode of treatment given to our patients, greater disease progression was observed in the non Ht 1 subgroup than in the subgroups with the Ht 1 haplotype, although the difference was not statistically significant (Table 4). These data suggest that a dose readjustment in the drugs given may be required according to

the different genotypes and haplotypes. In addition, more specific drugs that interact with *STAT4* could be given in addition to regular immunosuppressive regimens, especially in patients with GG genotype at rs3024912 SNP and the major Ht 1 haplotype.

The interpretation of our study results is limited because the patients were recruited from just one center in Taiwan. Our results suggest a significant role of *STAT4* polymorphisms in the risk of developing MGN of Taiwan. To the best of our knowledge, this is the first report on *STAT4* polymorphisms in MGN patients. However, the identification of *STAT4* 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.

In summary, our study firstly demonstrated the different genotype distribution between normal controls and patients with MGN of *STAT4* gene. The data show that *STAT4* gene is one of an important inflammatory related gene and may be associated with renal deterioration in MGN patients.

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Figure legend:

Figure 1. Map of STAT4 (rs3024912, rs3024908, rs3024877) located within Chromosome 2q32.3 region (191,894,302-192,016,322 bp).

Figure 2. The log-rank test was used for (A) renal and (B) patients survival analysis of 136 MGN patients with/without the Ht1 haplotype of the SATA4 gene.

Table 1

Genotypic and allelic frequencies of *SATA4* genetic polymorphisms in MGN patients and controls.

dbSNP ID		MGN patients	Controls	*p* value	OR (95% CI)
rs3024912					
	Genotype	**(N =136)**	**(N = 264)**		
	GG	30 (0.22)	71 (0.27)	0.388[a]	
	GT	75 (0.55)	127 (0.48)		
	TT	31 (0.23)	66 (0.25)		
	Allele frequency				
	G	137 (0.50)	259 (0.49)	0.725	1.05(0.79-1.41)
	T	135 (0.50)	269 (0.51)		1
rs3024908					
	Genotype	**(N =137)**	**(N = 265)**		
	AA	100 (0.73)	188 (0.71)	0.014[a]	
	AG	33 (0.24)	77 (0.29)		
	GG	4 (0.03)	0 (0)		
	Allele frequency				
	G	41 (0.15)	77 (0.15)	0.869	1.04(0.69-1.56)
	A	233 (0.85)	453 (0.85)		1
rs3024877					
	Genotype	**(N =138)**	**(N= 264)**		
	GG	36 (0.26)	61 (0.23)	0.797[a]	
	AG	70 (0.51)	138 (0.52)		
	AA	32 (0.23)	65 (0.25)		
	Allele frequency				
	G	142 (0.51)	260 (0.49)	0.552	1.09 (0.82-1.46)
	A	134 (0.49)	268 (0.51)		1

[a] Genotype distribution between patients and control were calculated by 2 x 3 chi-square test

CI, confidence interval; OR, odds ratio.

Table 2

Distribution of *SATA4* haplotype frequencies in MGN patients and controls.

Haplotype[a]	MGN patients (%)[b] (n=136)	Control (%) (n=264)	*p* value	OR (95% CI)
Ht 1 (G-A-A)	28.3%	33.7%	0.114	0.77 (0.56-1.05)
Ht 2 (T-A-G)	25.1%	24.1%	0.749	1.06 (0.75-1.48)
Ht 3 (G-A-G)	17.0%	13.3%	0.163	1.34 (0.89-2.00)
Ht 4 (T-A-A)	14.7%	14.3%	0.891	1.02 (0.67-1.54)
Ht 5 (T-G-G)	4.8%	7.7%	0.119	0.59 (0.31-1.31)

CI, confidence interval; OR, odds ratio.

[a]Order of single nucleotide polymorphisms comprising the *SATA4* haplotypes: rs3024912, rs3024908 and rs3024877.

[b]Percentages may not sum to 100% because of the presence of the presence of rare haplotypes[<5%] not presented here.

Table 3

Association between STAT4 genotypes and MGN patients with/without kidney failure.

dbSNP ID	Patients with MGN kidney failure		OR (95% CI)	p value	Adjusted OR (95% CI)[a]	p value
	no	yes				
rs3024912						
GG (n=27)	19 (17.3)	8 (38.1)	2.947(1.074-8.092)	0.041	3.255(1.155-9.176)	0.026
non GG (n=104)	91 (82.7)	13 (61.9)	1		1	
rs3024908						
AA (n=96)	82 (73.9)	14 (66.7)	0.707(0.260-1.927)	0.498	0.635(0.227-1.773)	0.386
non AA (n=36)	29 (26.1)	7 (33.3)	1		1	
rs3024877						
GG (n=35)	29 (25.9)	6 (28.6)	1.145(0.406-3.229)	0.798	1.350(0.461-3.957)	0.585
non GG (n=98)	83 (74.1)	15 (71.4)	1		1	

The Logistic Regression test was used

[a] Adjusted OR after controlling Gender and Follow-up period

Table 4

Comparison of the clinical features of MGN patients with/without the major Ht1 haplotype of STAT4 gene.

	Ht 1 (N = 78)	**non Ht 1** (N =58)	***p* value**
Age of biopsy (yrs)[a]	51.01 ± 17.79	56.11 ± 15.96	0.089
Duration of follow-up	6.6 ± 5.48	5.69 ± 4.55	0.311
BMI (Kg/M^2)[a]	24.66 ± 3.62	25.03 ± 3.42	0.547
Mean BP (mmHg)[a]	99.84 ± 15.93	101.77 ± 11.24	0.436
Albumin (gm/dl)[a]	2.53 ± 0.63	2.47 ± 0.62	0.583
Cholesterol (mg/dl)[a]	327.91 ± 132.29	320.55 ± 123.18	0.744
Triglyceride (mg/dl))[a]	202.61 ± 144.88	243.57 ± 165.39	0.132
Baseline serum Cr	1.42 ± 1.53	1.49 ± 1	0.746
Baseline DUP (gm/day)[a]	7.78 ± 11.91	7.94 ± 5.6	0.925
Baseline CCr (ml/min)[a]	85.7 ± 42.29	73.19 ± 37.46	0.080
Serum Cr at latest	2.68 ± 4.02	3 ± 3.01	0.617
Latest DUP (gm/day)[a]	2.47 ± 3.63	4.05 ± 5.37	0.064
Latest CCr (ml/min)[a]	64.2 ± 43.55	47.07 ± 34.9	0.017
Cardiovascular events	14 (18.7)	15 (25.9)	0.319
Other vascular events	18 (24.0)	15 (25.9)	0.805
Hematuria (%)	50 (66.7)	35 (60.3)	0.452
Lower leg edema (%)	64 (85.3)	54 (93.1)	0.160
Proteiuria ≧ 3.5 g/day	71 (94.7)	54 (93.1)	0.728
Malignancy (%)	7 (9.3)	4 (6.9)	0.755
Disease Progression (%)	34 (45.3)	28 (48.3)	0.736

BMI: body mass index; MBP: mean blood pressure; DUP: daily urinary protein excretion; CCr: creatinine clearance

[a] Data were presented as Mean ± SD (standard deviation)

[b] Cardiovascular events including: unstable angina, coronary artery disease, ischemic heart disease.

[c] Other vascular events including: renal artery or vein thrombosis, deep vein thrombosis and cranial vascular events

Table 5

STAT4 major Ht1 haplotype distribution and severity of pathological findings

	Ht 1	**non Ht 1**	***p* value**
Histological Stage	(N =60)	(N = 47)	
1	19 (31.7)	8 (17.0)	0.126
2	29 (48.3)	26 (55.3)	
3	8 (13.3)	10 (21.3)	
4	4 (6.7)	1 (2.1)	
5	0 (0)	2 (4.3)	
Global sclerosis*	(N = 68)	(N =50)	
0	36 (52.9)	19 (38.0)	0.249
1	20 (29.4)	21 (42.0)	
2	12 (17.6)	10(20.0)	
Tubule-interstitial fibrosis	(N =67)	(N =50)	
0	47 (70.1)	29 (58.0)	0.281
1	14 (20.9)	17 (34.0)	
2	6 (9.0)	4 (8.0)	
Intima fibroplasia of vess	(N = 65)	(N =50)	
0	49 (75.4)	35 (70.0)	0.525
1	13 (20.0)	10 (20.0)	
2	3 (4.6)	5 (10.0)	

The Chi-square test was used

*A semiquantitative scoring system was adopted, using a scale of 0 (absent), 1 (mild: <25%) and 2 (moderate to severe: >25%) for the assessment under the light microscopy.

Figure(s)

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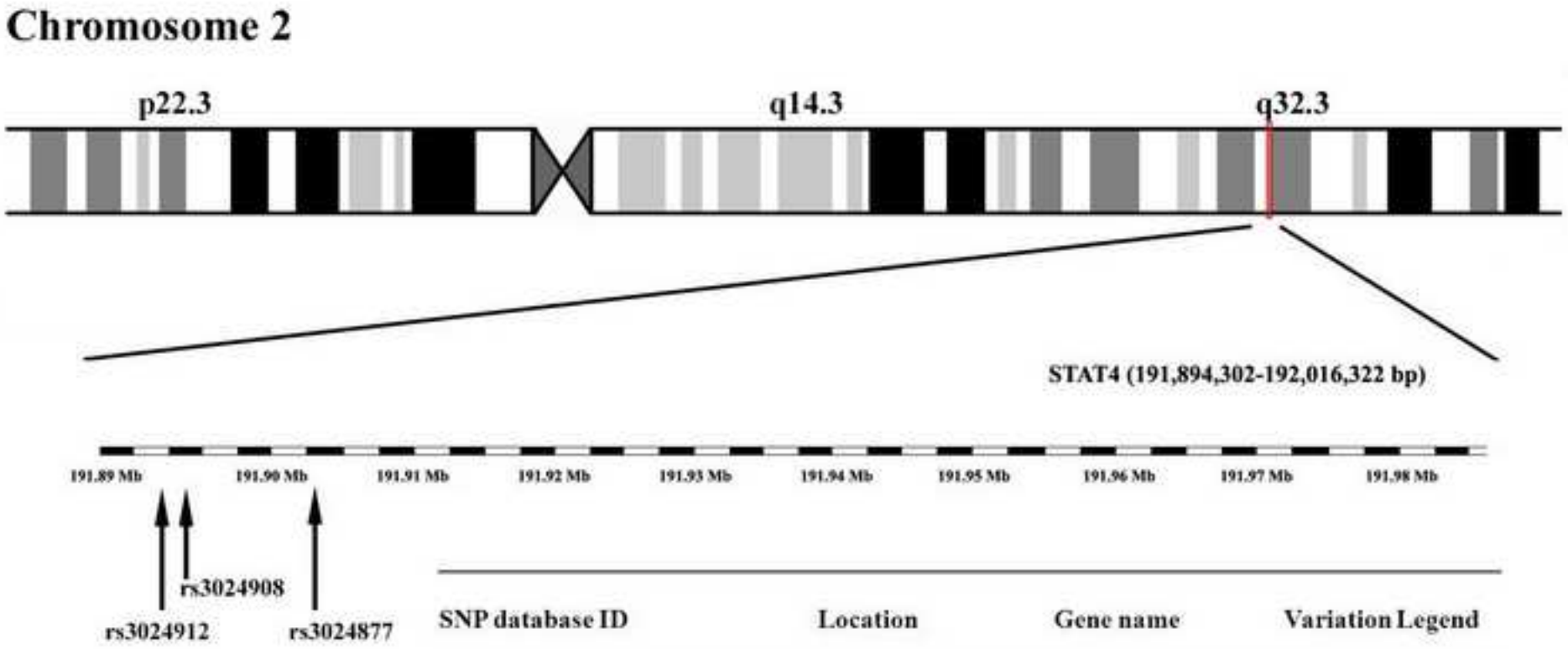

SNP database ID	Location	Gene name	Variation Legend
rs3024912	2:191893087	STAT4	3'UTR (G/T)
rs3024908	2:191894141	STAT4	3'UTR (A/G)
rs3024877	2:191904889	STAT4	intron 15 (A/G)

Figure(s)

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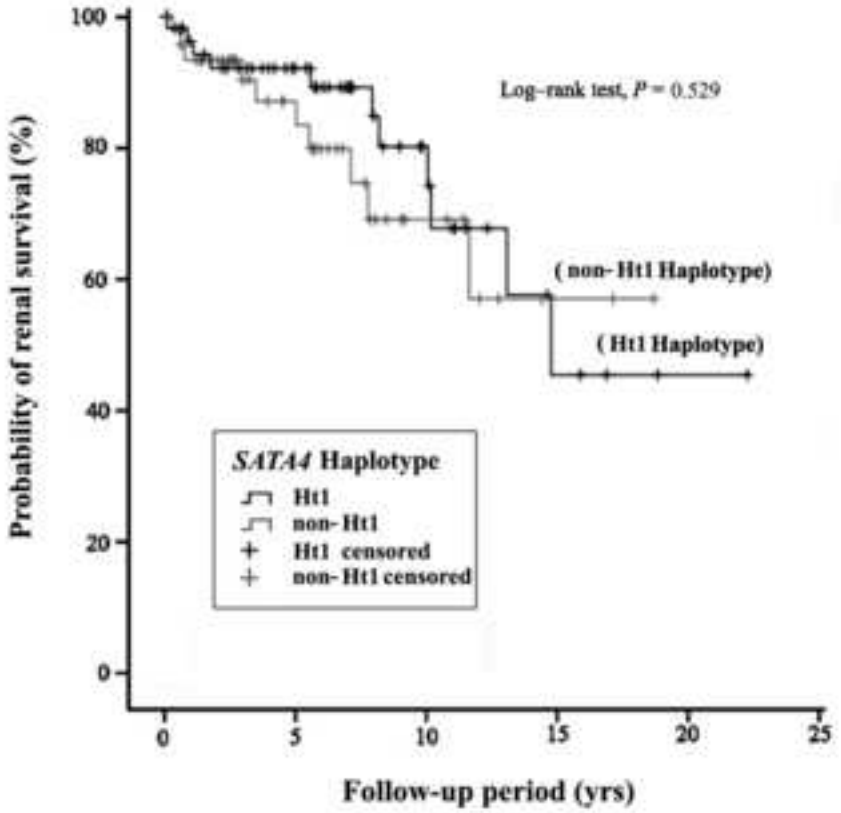
(A)



(B)