RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2013.39.625

ScienceAsia 39 (2013): **625–630**

Association between *MYH9* gene polymorphisms and membranous glomerulonephritis patients in Taiwan

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Received 5 Feb 2013
Accepted 22 Oct 2013

ABSTRACT: Membranous glomerulonephritis (MGN) is a common cause of idiopathic nephrotic syndrome in adults end-stage renal disease in 25% of patients. *MYH9* gene polymorphisms have been reported to be associated with several types of renal diseases. The objective of this study was to clarify the relationship between *MYH9* gene polymorphisms and the pathogenesis of MGN. We investigated *MYH9* gene polymorphisms (rs7078 and rs12107) and their association with MGN susceptibility in 400 Taiwanese individuals (135 MGN patients and 265 healthy controls). The results revealed a statistically significant difference in allele frequency distribution at the rs12107 between MGN patients and the control group ($p = 0.04$). In addition, individuals with the AA genotype at the rs12107 SNP who become MGN patients may have a higher risk of kidney failure than other MGN patients (adjusted odds ratio: 1.63; 95% confidence interval: 1.08–2.48, $p = 0.02$). A C-A haplotype was susceptible for development of MGN. Our data show that *MYH9* (rs12107) polymorphism may be the underlying cause of MGN; hence the polymorphism examined in this study warrant further investigation.

KEYWORDS: kidney disease

INTRODUCTION

Membranous glomerulonephritis (MGN) is the prime cause of nephrotic syndrome, accounting for approximately 40% of the adult cases[1]. It is characterized by basement membrane thickening and subepithelial immune deposits without cellular proliferation or infiltration[2]. Previous studies have confirmed MGN as a cause of chronic kidney disease and as a final result of end-stage renal disease (ESRD)[3]. Taiwan has the highest prevalence of ESRD worldwide and MGN may be one cause[4–6]. Thus the study of its inflammatory factors can help elucidate and prevent ESRD.

MGN is an immune-complex mediated disease, as evidenced by the presence of immunoglobulins and complement components in the capillary walls, and by the morphological and immunopathological similarities between experimental MGN and other immunological glomerular diseases[7]. Although a recent report has shown the M-type phospholipase A2 receptor to be the major target antigen, the aetiology and origin of the antigens that cause MGN remain unclear. The deposits may derive from circulating immune complexes formed in situ, or from previously deposited foreign antigens[8]. Although MGN is a multifactorial disease, an inflammatory pathway might play an important role in the pathogenesis of MGN[7,9].

MYH9-related disorders (*MYH9*-RD) are inherited in an autosomal dominant manner and are characterized by congenital thrombocytopenia and large platelets. These disorders are associated with the development of progressive nephropathy during infancy or adult life, sensorineural deafness, and presenile cataract[10,11].

The *MYH9* gene encodes the nonmuscle myosin IIA and is expressed in glomerular podocytes and mesangial cells[12,13]. The *MYH9* haplotypes show

Table 1 Demographics of MGN patients and controls.

Variables	MGN patients		Controls	
	Male	Female	Male	Female
Subjects (n)	90	45	155	110
Age (y)	55.8 ± 17.3	49.1 ± 16.8	53.5 ± 5.0	55.1 ± 5.2
Height (cm)	168.4 ± 6.9	156.1 ± 5.9	172.0 ± 4.7	159.7 ± 5.1
Weight (kg)	67.0 ± 10.0	59.7 ± 10.1	69.6 ± 11.2	54.1 ± 9.3
Body mass index (kg/m^2)	24.9 ± 3.2	24.6 ± 4.0	23.5 ± 3.6	22.2 ± 3.3

Values are expressed as mean $\pm$ SD. Respective values for each group were not significantly different.

replicated association with risk and protection. They have been found to be associated with kidney disease in African Americans and European Americans [14–17], and *MYH9* was also found to influence kidney function in Europeans [18]. Although the nephropathy associated with *MYH9* is markedly attenuated after accounting for the coding variants in *APOL1*, three groups have observed independent *MYH9* association with non-diabetic nephropathy [16]. Low associations were reported between *MYH9* and type 2 diabetes associated with ESRD in African Americans [16]. This may be explained by the fact that a subset of the patients thought to have diabetic nephropathy had focal segmental glomerulosclerosis with coincident type 2 diabetes [19].

Currently, 44 *MYH9* mutations have been reported [20], and these may involve either the N-terminal motor domain or the C-terminal tail domain of the *MYH9* gene encoding for the heavy chain of non-muscle myosin-IIA. Genotype-phenotype correlation studies have shown that patients with mutations affecting the C-terminal tail domain (TD) have a more severe thrombocytopenia and higher incidence of nephropathy and deafness than those with mutations involving the N-terminal motor domain (MD) [11]. The purpose of this study was to examine the 3′-UTR polymorphisms of the *MYH9* gene and their association with MGN in Taiwanese patients.

MATERIALS AND METHODS

Study population

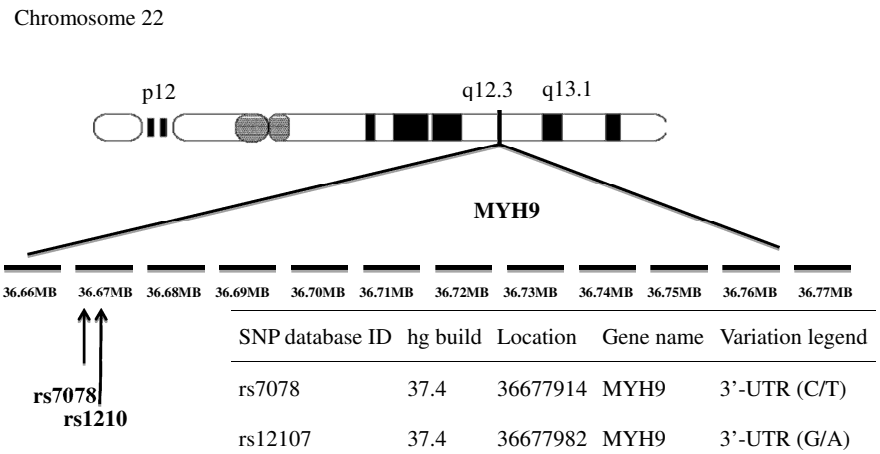
We recruited 135 patients with prior renal biopsy-proven MGN and 265 healthy controls from Taichung Veterans General Hospital during the period from 1982–2008. Those with malignant and chronically infectious diseases like hepatitis B and C, lupus nephritis, or drug-induced secondary MGN were excluded. As described previously [21], patient traits such as demographic variables, general data (e.g., gender, age of onset, body mass index, blood pressure) (Table 1), medical information (e.g., duration of follow-up, albumin, cholesterol), and vascular events (cardiovascular disease and peripheral vascular events) were reviewed for analysis. All participants signed an informed consent form. The study was approved by the institutional review board of the hospital (VGHTC IRB No. C08159). Treatment modality, either supportive or aggressive with immunosuppressants, was selected based on the decision of the treating physician. Supportive therapy usually included diuretics, angiotensin-converting enzyme inhibitors, and/or angiotensin II receptor blockers, depending on the symptoms of the patients. Immunosuppressive therapies included the following regimens: (a) prednisolone 1 mg/kg/day alone; (b) a 6-month course of corticosteroids alternated with chlorambucil at a dosage of 0.2 mg/kg/day every other month [22], or cyclophosphamide 1.5–2.0 mg/kg/day; and (c) cyclosporine A (CyA, Neoral, Norvatis AG, Basel, Switzerland) at 3–5 mg/kg/day with or without prednisolone.

Response and outcome

Based on previous reports, response to therapy was defined as the follows: (a) no response; (b) partial remission: proteinuria reduction of more than 50% or final proteinuria between 0.2 and 2.0 g/day; and (c) complete remission: proteinuria less than 0.2 g/day. Progression of renal disease was defined as a doubling of baseline serum creatinine values or entering ESRD. ESRD was defined as a patient requiring renal replacement therapy [21].

SNP selection and genotyping

Two SNPs in *MYH9* were chosen for genotyping based on strong evidence of an association with kidney disease in prior studies and subsequent detailed evaluation of the *MYH9* gene region and haplotypes [15, 16]. The SNPs of rs7078 and rs12107 are in the *MYH9* L1 risk haplotype previously associated with hypertension-associated ESRD [23]. Genomic DNA was extracted from peripheral blood leukocytes (Genomic



SNP database ID	hg build	Location	Gene name	Variation legend
rs7078	37.4	36677914	MYH9	3'-UTR (C/T)
rs12107	37.4	36677982	MYH9	3'-UTR (G/A)

Fig. 1 Map of *MYH9* located within chromosome 22q12.3 region (36 677 323–36 784 063 bp).

DNA kit, Roche). The genotypes of two SNPs (rs7078 and rs12107) at chromosome 22 positions 36 677 914 (3′-UTR) and 36 677 982 (3′-UTR) in the *MYH9* gene (Fig. 1) were performed using the SNP genotyping assay (Applied Biosystems Inc., Foster City). The primers and probes used to detect SNPs were from the ABI Assays-on Demand kit. Reactions were performed according to the manufacturer's protocol. Briefly, PCR was performed in the presence of 2× TaqMan Universal PCR Master Mix, assay mix and genomic DNA (15 ng). The fluorescence signal was detected by the ABI Prism 7900 Real Time PCR System.

Statistical analysis

Chi squared and Fisher's exact tests were used to determine statistically significant differences in allele/genotype frequencies between the case and control groups. Allelic frequencies were expressed as the percentage of total alleles. The Hardy-Weinberg equilibrium was tested for each marker using χ^2-test. Odds ratios (ORs) and 95% confidence intervals (CIs) were derived by logistic regression to correlate *MYH9* alleles/genotypes with MGN susceptibility. All data were analysed with SPSS Version 15.0 (SPSS Inc., Chicago, Illinois, USA). A p-value < 0.05 was considered statistically significant. Haplotypes were determined using the Bayesian statistical method available in the program Phase 2.1.32.

RESULTS

A statistically significant difference was found in the polymorphisms of the *MYH9* gene rs12107 G/A (3′-UTR) between the control group and patients with MGN ($p = 0.021$) (Table 2). The frequency of the 'AA' genotype was higher in patients with MGN (54%) than in the control group (42%). Compared with the 'GG+AG' genotype, the OR of 'AA' was 1.63 (95% CI = 1.08–2.48). The allelic frequency of 'A' was higher in patients with MGN (74%) than in the controls (67%). The OR for the 'A' allele was 1.39 (95% CI = 1.01–1.93, $p = 0.045$). The *MYH9* gene rs12107 G/A (3′-UTR) polymorphisms between controls and patients with MGN compared to that of the 'AG' genotype, the OR of 'AA' was 1.64 (95% CI = 1.06–2.53) and the OR of 'GG' was 1.02 (95% CI = 0.44–2.36). The data show a non-statistically significant difference in this comparison. The distributions of rs11703176 C/A (3′-UTR), and rs7078 C/T (3′-UTR) polymorphisms were also shown. There were no significant differences in genotype and allele frequency between the patient group and the control group. In addition, we used Hardy-Weinberg equilibrium (HWE) testing for data quality control. The results showed that the rs7078 and rs12107 SNPs are under the null hypothesis of no departure from HWE (Table 2).

Furthermore, we examined the relationship between *MYH9* (rs12107) AA and non-AA genotypes with stage, clinical, and biochemical manifestations in MGN patients. The results showed no significant differences between the stage, clinical, and biochemical manifestations in MGN patients with *MYH9* SNPs genotype and allele distribution (Table 3).

A haplotype analysis was performed to evaluate the *MYH9* haplotype associated with MGN patients in Taiwan. The risk alleles of two SNPs comparing this haplotype were evaluated (rs7078 and rs12107). By comparing haplotype frequency between the patient and the control groups, we were able to show the

Table 2 Genotypic and allelic frequencies of *MYH9* genetic polymorphisms in MGN patients and control.

dbSNP ID	Genotype	Patient with MGN	Control	OR (95% CI)		p value
rs7078		Total =134 (%)	Total=265 (%)			
	CC	115(85.8)	221(84.4)	1.15(0.64–2.07)		0.64
	CT	19(14.2)	42(15.6)	Ref		
	TT	0(0)	0(0)			
	Allele frequency					
	C	249(92.9)	484(92.4)	1.14(0.62–2)		0.65
	T	19(7.1)	42(7.6)	Ref		
rs12107		Total =135 (%)	Total=265 (%)			
	GG	9(6.7)	22(8.3)	1.02(0.44–2.36)		0.069[a]
	AG	53(39.3)	132(49.8)	Ref		
	AA	73(54)	111(41.9)	1.64(1.06–2.53)	1.63(1.08–2.48)	0.021
	GG+AG	62(46)	154(58.1)		Ref	
	Allele frequency					
	G	71(26.3)	176(33.2)	Ref		0.045
	A	199(73.7)	354(66.8)	1.39(1.01–1.93)		

CI, confidence interval; OR, odds ratio.

[a] Genotype distributions between patients and control were calculated by 2×3 chi-squared test.

Table 3 Characteristics of clinical parameters between AA and non AA (rs12107) polymorphism of *MYH9* gene in patients with MGN.

Clinical parameters	*MYH9* (rs12107)		
	AA ($n = 70$) (%)	non AA ($n = 60$) (%)	p value
Male	37 (53)	37 (62)	0.31
Female	33 (47)	23 (38)	N/A
Age (years)	53 ± 16	53 ± 18	0.98
Weight (kg)	64 ± 11	64 ± 10	0.83
Height (cm)	160 ± 7	161 ± 8	0.72
BMI (kg/m^2)	25 ± 4	25 ± 3	0.62
Systolic BP (mmHg)	136 ± 18	135 ± 23	0.78
Diastolic BP (mmHg)	82 ± 11	84 ± 15	0.37
Mean BP (mmHg)	100 ± 12	101 ± 17	0.68
Cholesterol (mg/dl)	334 ± 111	316 ± 148	0.44
Triglyceride (mg/dl)	242 ± 180	202 ± 118	0.15
Positive proteinuria	66 (94)	56 (93)	1.00
Native proteinuria	4 (6)	4 (7)	
Kidney function (normal)	58 (83)	51 (85)	0.74
Kidney function (failure)	12 (17)	9 (15)	
Grade 1	14 (25)	13 (27)	0.97
Grade 2	30 (54)	24 (49)	
Grade 3	8 (14)	9 (18)	
Grade 4	3 (5)	2 (4)	
Grade 5	1 (2)	1 (2)	

N/A: not applicable.

haplotype frequency distribution of the *MYH9* gene in 3 genetic variants (Table 4). Haplotype 1 (C-A) was the common haplotype in MGN patients (74%) and of those in the control group (67%), with $p = 0.041$ and OR $= 1.41$ (95% CI 1.01–1.95). There was a statistical difference between MGN patients and the control group. Haplotype 2 (C-G) was present at a frequency of 19% in MGN patients and 25% in the control group, with $p = 0.050$ and OR $= 0.69$ (95% CI 0.48–0.99). There was non-statistical difference between MGN patients and the control group.

DISCUSSION

MGN is considered to be a multiple factorial disease with immunologic expressions that may occur in people who are genetically susceptible[21,24]. Polymorphisms in cytokine gene sequences known to be involved in the pathogenesis of MGN are potential markers of disease susceptibility. *MYH9* encodes the protein non-muscle myosin heavy chain, class II, and the isoform type A in eukaryotic cells. The gene is approximately 110 kb with 41 exons and is highly conserved among a number of mammalian species and similar to other non-muscle myosin isoforms[25]. The protein is abundantly expressed in the kidney, liver, and platelets. *MYH9*-related diseases encompass a series of autosomal dominant macrothrombocytopenias which were previously considered to be distinct disorders. These include May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome, which derive from mutations in the *MYH9* gene encoding for the heavy chain of nonmuscle myosin IIA. Our results show that the effecting *MYH9* SNP (rs12107) lies in the 3′ untranslated region (3′-UTR) in MGN patients. Genotype-phenotype correlation studies have shown that patients with mutations affecting the C-terminal TD have more severe cases of thrombocytopenia and a higher incidence of nephropathy and deafness than those with mutations involving the N-terminal MD domain[11,20].

Table 4 Distribution of *MYH9* haplotype frequencies in the patients with MGN and controls.

Haplotype[a]	Patient with MGN (%) ($n = 135$)[b]	Control (%) ($n = 265$)	OR (95% CI)	p value
1(C-A)	74	67	1.41 (1.01–1.95)	0.04
2(C-G)	19	25	0.69 (0.48–0.99)	0.05
3(T-G)	7.1	8	0.89 (0.50–1.56)	0.70

[a] Order of single nucleotide polymorphisms comprising the *MYH9* haplotypes: rs7078, rs12107.
[b] Percentages may not sum to 100% because of the rare haplotypes ($< 5\%$) not presented here.
CI, confidence interval; OR, odds ratio.

A lack of association between *MYH9* and diabetic kidney disease does not disprove the hypothesis that common mechanisms exist for the progression of all forms of chronic kidney disease. This study suggests that at least some mechanisms of ESRD progression are unique to specific diseases. *MYH9* is critical to the progression of nondiabetic ESRD but not to the progression of diabetic ESRD. *MYH9* may also have differential effects on the progression towards ESRD, depending on the type of diabetes [14].

The major limitation of this study is the small sample size of the MGN patients. In the present study, the statistical power (assuming $a = 0.05$) reached 52% for subjects with or without MGN for the *MYH9* gene on chromosome 22q. We have compared the allele frequencies of these SNPs in healthy controls among the Han Chinese population in Taiwan. The AA allele frequencies of rs12107 SNP located in the *MYH9* gene was higher among the Han Chinese population in Taiwan. Hence future studies with a larger number of subjects or subjects from different ethnic backgrounds will be necessary to determine whether these findings can be replicated. Compared with the control group, the C-A haplotype appears to be a susceptibility factor for the development of MGN in our Taiwanese cohort.

This is the first study to identify *MYH9* SNP associations with MGN in people in Taiwan. Our study demonstrates the various genotype distributions of the *MYH9* gene among healthy controls and patients with MGN. The data show that the *MYH9* gene is a critical gene which may be associated with renal deterioration in MGN patients.

Acknowledgements: This work is supported in part by Taiwan Department of Health Clinical Trial and Research Centre of Excellence (DOH101-TD-B-111-004), China Medical University (CMU100-S-06) and China Medical University Hospital (DMR-101-041) in Taiwan. The first author fellowship is supported by China Medical University (CMU101-AWARD-01), Taiwan. Y-T Chen, C-H Chen, C-H Yen, and S-Y Chen contributed equally to this paper.

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