ORIGINAL RESEARCH

Clinical Predictive Value of Phospholipase A2 Receptor Gene Polymorphism Combined with Subclass of Immunoglobulin G in Renal Tissues for Membranous Nephropathy

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ABSTRACT

Context • Idiopathic membranous nephropathy (IMN) is a common pathologic type of nephrotic syndrome, and the level of the M-type phospholipase A2 receptor (PLA2R) antibody can serve as one index for predicting its progression and prognosis. However, patients with the same level can show great differences in their responses and prognoses.

Objectives • The study aimed to explore the relationship between a PLA2R gene polymorphism combined with an immunoglobulin G (IgG) subclass in renal tissues and patients' responses to immunosuppressive therapy, to determine the clinical prognosis for IMN patients.

Design • This is a prospective study. Patients with new onset membranous nephropathy who need treatment were selected and grouped according to the curative effect after 6 months of treatment.

Setting • The study took place at the First Affiliated Hospital of Ningbo University, Ningbo, China.

Participants • Participants were 60 patients with IMN, who had been admitted in the hospital between January 1, 2021 and June 30, 2022.

Intervention • Participants first received standard immunosuppressive therapy for six months. The research team then clinically divided participants into two groups: (1) a remission group with 32 participants and (2) a nonremission group with 28 participants.

Outcome Measures • The research team: (1) compared the groups, summarizing the demographic and clinical differences between the groups, (2) compared the PLA2R antibody titers at baseline and postintervention between the groups, (3) analyzed the genotyping of the PLA2R single nucleotide polymorphisms (SNPs) rs35771982 and rs4664308 loci as well as the human leukocyte antigen (HLA)-DQA1 SNP rs2187668 locus, and (4) compared the subclass IgG and PLA2R depositions in the renal tissues between the groups.

Results • Compared with the remission group, the nonremission group included significantly more males ($P < .05$), was significantly older ($P < .05$), had significantly more participants with a BMI of >25 ($P < .05$), and included significantly more participants with a positive IgG3 ($P < .01$) than the remission group. The remission group's PLA2R antibody titers at baseline and postintervention weren't significantly different from those of the nonremission group. Postintervention, 24 participants in the remission group had a negative conversion of PLA2R antibodies, and 22 in the nonremission group had a negative conversion. The genotyping of the PLA2R SNP rs4664308 and the HLA-DQA1 SNP rs2187668 loci showed no relationship to the remission rate. The GC genotype on the PLA2R SNPrs35771982 locus may be a risk factor for a poor prognosis for IMN patients. Moreover, the patients with a positive IgG3 in the renal tissues and the GC genotype on the PLA2R SNPrs35771982 locus exhibited a poor response to immunosuppressive therapy and could need intensive treatment.

Conclusions • The PLA2R gene polymorphism combined with the IgG subclass can predict the sensitivity of IMN patients to immunosuppressive therapy. (*Altern Ther Health Med.* 2023;29(7):418-423).

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Idiopathic membranous nephropathy (IMN) is a common pathologic type of nephrotic syndrome in adult patients. In 2009, Beck et al's study on IMN found that the M-type phospholipase A2 receptor (PLA2R) has been of epochal significance in the study of IMN, which found that autoantibodies against PLA2R are present in the serum of about 70% of IMN patients.[1] The *Kidney Disease: Improving Global Outcomes (KDIGO)* guideline in 2020 indicated that the PLA2R antibody level and the trend of changes were important monitoring indexes for risk stratification and immunotherapy guidance.

PLA2R Antibody Titer

The level of the PLA2R antibody titer can serve as an index for predicting the progression and prognosis of IMN.[2-4] The higher the PLA2R antibody is, the lower the possibility is that a spontaneous remission will occur.

For patients who attain remission after immunosuppressive therapy, the PLA2R antibody either converted from positive to negative or the antibody titer decreased. However, most IMN patients don't attain remission until several months after receiving a negative finding for the PLA2R antibody. If the antibody converts to positive or the antibody titer increases, relapses of nephrotic syndrome can occur.

Both the clinical manifestations of IMN and the responses to therapy can be very different for different patients. Although it might seem that clinicians could base the administration of immunosuppressive therapy on the level of the PLA2R antibody titer, they have found that no relationship exists for some patients between IMN's sensitivity to immunosuppressive medications and the PLA2R titer. Patients with same PLA2R antibody titer can show great differences in their responses and prognoses.

Attendees at the International Society of Nephrology's (ISN's) International World Nephrology Congress (WNC-2019) discussed some issues that remained unsettled by the conference's end.[5] Van de Logt et al at the University of Manchester in the UK published a review that concentrated on those issues.[5] Those researchers believed that further studies on genetic factors and T-cell and B-cell epitopes should occur to identify the risk factors for IMN progression, thereby developing an optimized and targeted treatment strategy.

Immunoglobulin G (IgG)

Van de Logt et al also indicated that the subclass of IgG and the specialization of the PLA2R antigenic epitope might be more-specific predictive indexes for refractory IMN.[5] The IgG subclass of antibody deposited in the capillary walls of the glomerulus, is mainly IgG1 at the disease's early stage, accompanied by deposition of the iolinitiation factor Clq, a classical pathway of complement activation. With the progression of the pathological stage, the Clq signal gradually weakens, and the IgG4 signal becomes increasingly strong, indicating a possible IgG-subclass conversion in the course of disease's evolution.[6] The IgG deposited in the capillary wall of the glomerulus is mainly IgG1 subtype in the early stage of the disease, and gradually changes to IgG4 as the disease progresses.

Although the IgG4 subclass is the main anti-PLA2R antibody in serum, different amounts of IgG1 and IgG3 exist for the classical pathway of activation of IMN in the serum of patients and can occur even for some patients in the absence of IgG4. Researchers can't eliminate the possibility of classical-pathway activation by IgG1 or IgG3 at a certain stage of IMN[7].Moreover, the characteristics of IgG-subclass deposition may influence the severity and prognosis of renal lesions.

Gene Polymorphism

Some studies have found that PLA2R gene's single nucleotide polymorphism (SNP) locus and gene polymorphism can affect the progression of IMN by regulating the formation of the PLA2R antibody and the expression of PLA2R on the surface of podocytes.[8-10] In 2016, Cheng et al demonstrated that a mutation at the PLA2R gene SNP rs35771982 locus is the risk factor for IMN onset.[11]

Moreover, after the mutation of the G/C genotype, aspartic acid can replace histidine. This leads to a structural change in the C-type, calcium-dependent, lectin domain; exposure of the PLA2R antigenic epitope; and formation of specific anti-PLA2R antibodies, further promoting the development and progression of IMN and affecting its prognosis.

Current Study

An in-depth study could be beneficial to a better understanding of the pathogenesis of IMN, to make risk assessments and determine individualized therapy for patients. The current study aimed to explore the relationship between a PLA2R gene polymorphism combined with an IgG subclass in renal tissues and patients' responses to immunosuppressive therapy, to determine the clinical prognosis for IMN patients.

METHODS

Participants

The research team performed a prospective study for newly diagnosed patients requiring immunotherapy which took place at the First Affiliated Hospital of Ningbo University, Ningbo, China. Potential participants were 60 patients with IMN, who had been admitted in the hospital between January 1, 2021 and June 30, 2022. These patients were newly diagnosed patients requiring immunotherapy in our hospital, and the author was the attending physician of these patients. After treatment with the guideline regimen, the patients were followed up in the outpatient department.

The study included potential participants if they: (1) had received a diagnosis of new-onset membranous nephropathy, (2) were >18 years of age, (3) had received no glucocorticoids or other immunosuppressive therapy before a renal biopsy, and (4) had initiated immunosuppressive therapy in accordance with the 2020 *Kidney Disease: Improving Global Outcomes* guidelines .

The study excluded potential participants if they had systemic lupus erythematosus or secondary membranous nephropathies, such as hepatitis-B-associated glomerulonephritis or tumor-associated glomerulonephritis.

All patients in this study were newly diagnosed nephrotic syndrome patients. After screening, all patients signed informed letters with them, long-term outpatient and telephone follow-up were completed, and no patients were lost to follow up.

This study was approved by the ethics committee of the hospital, in line with the Declaration of Helsinki, and patients were informed. All patients signed the informed consent form.

Procedures

Immunosuppressive therapy. The research team drew 5 ml of whole blood from participants' peripheral veins at admission. The team then selected the patients needing

immunosuppressive therapy based on the guideline on management of membranous nephropathy, and then they all received a tolerable dose of angiotensin receptor blockers. For the detection of gene polymorphism, 5 ml of whole blood needs to be extracted and sent out, and informed postoperative extraction is signed after admission. The remaining tests involved in this paper are routine tests after admission, which are the same as those for other patients not enrolled, so they are not mentioned separately.

Groups. After observation for half a year, the research team divided participants into two groups: (1) the remission group, including both complete remission and partial remissions, and (2) the nonremission group.

Clinical and laboratory data. During the performance of the renal biopsies, the research team collected data, including that from the immunosuppressive therapy, laboratory indexes, pathological indexes, and about the status of remission. The demographic and clinical data included gender, age at biopsy, basic blood pressure, and body mass indexes (BMIs).

At baseline and postintervention, the team collected data from the evaluation of renal lesions, including serum creatinine (Scr), plasma albumin (Alb), 24h urine protein (24hUPro), and serum PLA2R antibody titer. The immune index referred to identification of participants' IgG3 subclasses from the testing of renal tissues.

DNA extraction. The above DNA extraction operation was sent to Shanghai Xiaoyan Biotechnology. DNA extracted from the whole blood, and used 5% ethylenediaminetetraacetic acid (EDTA) for anticoagulation. The team used the Ezup Column Blood Genomic DNA Purification kit (Shanghai Sangon Biotech (B518253, Shanghai, China), rapidly extracting the genomic DNA from the whole blood and then storing at -80°C.

PLA2R detection. The research team employed technology from Shanghai Xiaoyan Biotechnology to perform a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), to detect the genotyping and allele frequency of the PLA2R SNP rs4664308, rs35771982, and HLA-DQA1 SNP rs2187668 loci. The team used sequence analysis software to analyze the data. The team used synthesized primers from Shanghai Sangon Biotech:

Pathological testing. The research team fixed the tissue from the renal biopsies in 10% formalin and sent them to Guangzhou KingMed Diagnostics (Guangzhou, China) for staining with hematoxylin and eosin (HE), periodic Schiff-methenamine (PASM), periodic acid–Schiff (PAS), and Masson, within one week of paracentesis.

The team (Guangzhou Golden Domain) then used the direct immunofluorescence technique to detect the subclass IgG and PLA2R depositions in the renal tissues. The team also recorded the fluorescence intensity in a semiquantitative way and from negative to peak, indicated by 0 to 4+. The team defined fluorescence intensity ≥1+ is defined as positive, 3+ and 4+ = strongly positive.

Efficacy evaluation. The criteria for the evaluation of the immunosuppressive therapy were: (1) complete remission—urine proteins of <0.3 g/d, plasma albumin of >35g/L, the absence of edema, and a stable renal function; (2) partial remission (PR)—urine proteins of 0.3-3.5 g/d, plasma albumin of >30 g/L, the absence of edema, and a stable renal function; and (3) no remission (NR)—urine protein s of >3.5 g/d, plasma albumin of <30 g/l, and edema or aggravated renal function. The results for the remission group included both complete and partial remissions.

Outcome measures. The research team: (1) compared the groups, summarizing the demographic and clinical differences between the groups, (2) compared the PLA2R antibody titers at baseline and postintervention between the groups, (3) analyzed the genotyping of the PLA2R single nucleotide polymorphisms (SNPs) rs35771982 and rs4664308 loci as well as the human leukocyte antigen (HLA)-DQA1 SNP rs2187668 locus, and (4) compared the subclass IgG and PLA2R depositions in the renal tissues between the groups.

Primer Table

rs4664308-F	CTGCTGCCCTGTTCTACCAC
rs4664308-R	GACAGCCTCCATCCTCTCTG
rs35771982-F	TATGTGCATGGGAAATGCTG
rs35771982-R	GTGGGAGCCTGCTTTATCAC
rs2187668-F	TGCCCGTTTCTTTCTCTCAG
rs2187668-R	TGAAGAACAGGTAATTTGGGTTG

Table 1. PCR

Reagent name	Supplier	Number	Remark
Taq Plus DNApolysaccharase	Sangon Biotech	B600090	
10X PCR Buffer (Mg2+)	Sangon Biotech	B600017	
dNTP (10 mM)	Sangon Biotech	B500056	
The sterilization deionized water	Sangon Biotech	E607017	
primer DNA	Sangon Biotech	/	Homemade

Table 2. Electrophoresis and recovery

Reagent name	Supplier	Number
6X DNA Loading Dye	ThermoFisher	R0611
DNA Ladder Mix (100-10000bp)	ThermoFisher	SM0331
DNA Ladder Mix (100~3000bp)	Sangon Biotech	B500347
50X TAE	Sangon Biotech	B548101
agarose H	Sangon Biotech	A620014
SanPrepcolumntypeDNAGel recovery kit	Sangon Biotech	B518131
absolute ethyl alcohol	Sangon Biotech	A500737
isopropyl alcohol	Sangon Biotech	A507048
1X TE	Sangon Biotech	B548106

Table 3. DNA sequencing

Reagent name	Supplier	Number
BigDye Terminator v1.1	ThermoFisher	4336701
POP-7™ Polymer	ThermoFisher	4363785
HiDi Formamide	ThermoFisher	4311320
EDTA	Sangon Biotech	A500895
NaAc	Sangon Biotech	A500827
NaOH	Sangon Biotech	A620617

Table 4. DNA draw

Name of kit	Supplier	Number	Remark
Ezup column animal tissue genomic DNA extraction kit	Sangon Biotech	B518251	http://www.sangon.com/productImage/DOC/B518251/B518251_ZH_P.pdf
Ezup column blood genomic DNA extraction kit	Sangon Biotech	B518253	http://www.sangon.com/productImage/DOC/B518253/B518253_ZH_P.pdf
Ezup column plant tissue genomic DNA extraction kit	Sangon Biotech	B518261	http://www.sangon.com/productImage/DOC/B518261/B518261_ZH_P.pdf
Ezup column deep-processed sample genomic DNA extraction kit	Sangon Biotech	B518264	http://www.sangon.com/productImage/DOC/B518264/B518264_ZH_P.pdf
Ezup column oral test genomic DNA extraction kit	Sangon Biotech	B518268	http://www.sangon.com/productImage/DOC/B518268/B518268_ZH_P.pdf
Ezup column viral genomic DNA extraction kit	Sangon Biotech	B518267	http://www.sangon.com/productImage/DOC/B518267/B518267_ZH_P.pdf
Ezup column saliva and urine cause group DNA extraction kit	Sangon Biotech	B518266	http://www.sangon.com/productImage/DOC/B518266/B518266_ZH_P.pdf
Ezup column bacterial genomic DNA extraction kit	Sangon Biotech	B518255	http://www.sangon.com/productImage/DOC/B518255/B518255_ZH_P.pdf
Ezup column fungal genomic DNA extraction kit	Sangon Biotech	B518259	http://www.sangon.com/productImage/DOC/B518259/B518259_ZH_P.pdf
Ezup column yeast genomic DNA extraction kit	Sangon Biotech	B518257	http://www.sangon.com/productImage/DOC/B518257/B518257_ZH_P.pdf
Ezup column soil genomic DNA extraction kit	Sangon Biotech	B518263	http://www.sangon.com/productImage/DOC/B518263/B518263_ZH_P.pdf

Table 5. PCR Reaction System

Component	Concentration	Volume (μL)
Template DNA		1
Primer F	10 μM	1
Primer R	10 μM	1
dNTP (mix)	10 mM	1
Taq Buffer with $MgCl_2$	10×	2.5
Taq DNA polymerase	5 U/μL	0.2
Add ddH_2O to		25

Abbreviations: dNTP, deoxynucleoside triphosphates; $MgCl_2$, magnesium chloride

Table 6. PCR Reaction Condition

Number	Procedure	Temperature	Time
1	Initial denaturation	95°C	5 min
2	Denaturation	94°C	30 sec
3	Anneal; annealing temperature decreased 0.5°C per cycle	63°C	30 sec
4	Extension	72°C	30 sec
5	Cycles 2 to 4	10 cycles	
6	Denaturation	95°C	30 sec
7	Anneal	58°C	30 sec
8	Extension	72°C	30 sec
9	Cycles 6 to 8	30 cycles	
10	Renaturation and extension	72°C	10 min
11	Insulation	4°C	

Table 7. Comparison of Demographic and Clinical Characteristics of the Nonremission and Remission Groups (N=60). Age refers to the age at the time of the renal biopsy. For the degree of renal tubular atrophy, 0 = ≤5%, 1= >5% but ≤25%, 2 = >25% but ≤50%, and 3 = >50%. Regarding immunofluorescence, 0-4 points indicates fluorescence intensities of 0 to 4+.

Characteristics	Nonremission Group n = 28 n (%) Mean ± SD	Remission Group n = 32 n (%) Mean ± SD	*P* value
Gender			<.05[a] (.028)
Male	21 (75.00)	18 (56.25)	
Female	7 (25.00)	14 (43.75)	
Age, y	69 ± 13.67	44 ± 8.98	<.05 (.036)
Hypertension			>.05[a] (.067)
Yes	18 (64.29)	13 (40.62)	
No	10 (35.71)	19 (59.38)	
BMI index			<.05[a] (.004)
<25	8 (28.57)	21 (65.63)	
>25	20 (71.43)	11 (34.37)	
24 h urine protein, g	8.47 ± 2.43	7.98 ± 2.22	>.05 (0.698)
Alb, g/L	25.25 ± 6.87	27.09 ± 7.07	>.05 (.861)
Scr, μmol/L	73.45 ± 19.98	62.97 ± 19.78	>.05 (.664)
Degree of renal tubular atrophy			>.05 (.837)
0	16 (57.15)	18 (56.25)	
1	10 (35.71)	13 (40.62)	
2	2 (7.14)	1 (3.13)	
3	0 (0.00)	0 (0.00)	
PLA2R Immunofluorescence			>.05 (.860)
0	8 (28.57)	6 (18.75)	
1	7 (25.00)	10 (31.25)	
2	10 (35.71)	12 (37.50)	
3	3 (10.72)	4 (12.50)	

Characteristics	Nonremission Group n = 28 n (%) Mean ± SD	Remission Group n = 32 n (%) Mean ± SD	*P* value
IgG1 Immunofluorescence			>.05(.939)
0	0 (0.00)	0 (0.00)	
1	10 (35.71)	13 (40.62)	
2	14 (50.00)	14 (43.75)	
3	4 (14.29)	5 (15.63)	
IgG2 Immunofluorescence			>.05 (0.062)
0	6 (21.43)	12 (37.50)	
1	20 (71.43)	12 (37.50)	
2	2 (7.14)	8 (25.00)	
3	0 (0.00)	0 (0.00)	
IgG3 Immunofluorescence			<.01 (<.001)
0	2 (7.14)	26 (81.25)	
1	16 (57.15)	6 (18.75)	
2	8 (28.57)	0 (0.00)	
3	2 (7.14)	0 (0.00)	
IgG4 Immunofluorescence			>.05 (.127)
0	0 (0.00)	0 (0.00)	
1	4 (14.29)	12 (37.50)	
2	10 (35.71)	8 (25.00)	
3	14 (50.00)	12 (37.50)	

[a]$P < .05$, indicating that the nonremission group included significantly more males, was significantly older, had significantly more participants with a BMI of >25, and had significantly more participants with IgG3 than the remission group

Abbreviations: Alb, serum albumin; BMI, body mass index; IgG1, immunoglobulin G1; PLA2R, phospholipase A2 receptor; Scr, serum creatinine

Outcome Measures

Demographic and clinical characteristics. Age refers to the age at the time of the renal biopsy. For the degree of renal tubular atrophy, 0 = ≤5%, 1 = >5% but ≤25%, 2 = >25% but ≤50%, and 3 = >50%. Regarding immunofluorescence, 0-4 points indicates fluorescence intensities of 0 to 4+.

PLA2R antibody titers. Serum PLA2R levels in membranous nephropathy do not reflect the prognosis of immunosuppressive therapy.

PLA2R single nucleotide polymorphisms. PLA2R single nucleotide polymorphisms may be a way to predict the efficacy of immunosuppressive therapy in patients with membranous nephropathy.

Subclass IgG and PLA2R depositions. Patients with membranous nephropathy with IgG3 positive renal tissue may have poor immunosuppressive effect and need intensive treatment.

Statistical Analysis

The research team analyzed the data using the SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The team: (1) expressed the measurement data with a normal distribution as means ± standard deviations (SDs) and compared the groups using the t test and (2) expressed the counting data as numbers (N) and percentage (%) and used the Chi Square (χ^2) test for comparisons between groups. $P < .05$ indicated a statistically significant difference.

RESULTS

Participants

In the nonremission group, eight participants had accepted an initial treatment regimen of corticosteroids combined with cyclophosphamide, and 20 had accepted treatment with rituximab. In the remission group, 18 participants had accepted an initial treatment regimen of hormones combined with cyclophosphamide, and 14 had accepted treatment with rituximab.

The research team included and analyzed the data of 60 participants, including 39 males and 21 females (Table 8). After half a year of treatment, the nonremission group included 28 participants, and the remission group included 32 participants. The nonremission group included 21 males (75.00%) and seven females (25.00%), with a mean age of 69 ± 13.67.

The remission group included 18 males (56.25%) and 14 females (43.75%, with a mean age of 44 ± 8.98. In the nonremission group, eight participants had a BMI of <25 (28.57%) and 20 had a BMI of >25 (71.43%). In the remission group, 21 participants had a BMI of <25 (65.63%) and 11 had a BMI of >25 (34.37%). The nonremission group included significantly more males ($P < .05$), was significantly older ($P < .05$), and had significantly more participants with a BMI of >25 ($P < .05$) than the remission group.

Based on the IgG3 immunofluorescence, four participants in the nonremission group had an intensity of one, two had an intensity of 2, and three had an intensity of 3. In the nonremission group, 12 participants in the nonremission group had an intensity of one, eight had an intensity of 2, and 12 had an intensity of 3. The nonremission group included significantly more participants with a positive IgG3 than the remission group did ($P < .01$).

Table 8. Comparison of the Antibody Levels of Serum PLA2R at Baseline Before Immunosuppressive Therapy and Postintervention After Therapy Between the Nonremission and Remission Group (n = 60)

Timepoint	Nonremission Group n = 28 RU/mL Mean ± SD	Remission Group N=32 RU/mL Mean ± SD	*P* value
Baseline	120.58 ± 46.45	103.21 ± 35.43	>.05 (.626)
Postintervention	27.26 ± 6.65	21.87 ± 4.87	>.05 (.538)
Postintervention	**n (%)**	**n (%)**	***P* value**
Negative conversion of serum PLA2R	22 (78.57)	24 (75.00)	>.05 (.932)

Abbreviations: PLA2R, phospholipase A2 receptor.

Table 9. Relationship Between Locus of Gene Polymorphism for the PLA2R Gene SNP rs35771982 and the Remission Rate of Participants With IMN (n = 60)

Genotype	n	Complete or Partial Remission n (%)	No Remission n (%)	χ^2	*P* value
GG	32	22 (68.75)	10 (31.25)	6.903	.027
GC	20	8 (40.00)	12 (60.00)		
CC	8	2 (25.00)	6 (75.00)		

Abbreviations: IMN, ischemic monomelic neuropathy; PLA2R, phospholipase A2 receptor; SNP, single nucleotide polymorphism

Table 10. Relationship Between Locus of Gene Polymorphism for the PLA2R Gene SNP rs35771982 Combined With a Positive IgG3 in Renal Tissues and the Remission Rate of Participants With IMN (n = 60)

Genotype		n (%)	Complete or Partial Remission n (%)	No Remission n (%)	χ^2	*P* value
GG,	GG +IgG3+	4 (12.50)	1 (25.00)	3 (75.00)	2.078	.149
n = 32	GG+IgG3-	28 (87.50	21 (75.00)	7 (25.00)		
GC,	GC +IgG3+	14 (70.00)	4 (28.57)	10 (71.43)	1.200	.273
n = 20	GC+IgG3-	6 (30.00)	4 (66.67)	2 (33.33)		
CC,	CC+IgG3+	8 (100.00)	2 (25.00)	6 (75.00)		
n = 8	CC +IgG3-	0 (0.00)	0 (0.00)	0 (0.00)		

Abbreviations: IgG3, immunoglobulin G3; IMN, ischemic monomelic neuropathy; PLA2R, phospholipase A2 receptor; SNP, single nucleotide polymorphism.

Antibody Titers

Table 9 shows that the remission group's PLA2R antibody titers at baseline and postintervention weren't significantly

different from those of the nonremission group ($P > .05$). Postintervention, 24 participants in the remission group had a negative conversion of PLA2R antibodies, and 22 in the nonremission group had a negative conversion. Simultaneously, the PLA2R antibody level of other participants decreased to different degrees. The PLA2R turned negative in 24 of 32 patients in the response group and 22 of 28 patients in the non-response group.

Gene Polymorphism

The genotypes for the PLA2R SNP rs4664308 and HLA-DQA1 SNP rs2187668 loci had no relationship to remission rate (data not shown). The GC genotype at the PLA2R SNPrs35771982 locus may be a risk factor for a poor prognosis for IMN patients (Table 10).

Gene Polymorphism and Positive IgG3

Participants with a positive IgG3 in the renal tissues and the GC genotype on the PLA2R SNPrs35771982 locus exhibited a poor response to immunosuppressive therapy and could need intensive treatment.

DISCUSSION

The current study found that the remission group's PLA2R antibody titers after immunosuppressive therapy weren't significantly different from those of the nonremission group. Negative conversion of the PLA2R antibody occurred for 22 out of 28 participants in the nonremission group, with their condition not changing. Therefore, it's necessary to find a reliable index to predict the remission of membranous nephropathy.

The current study found that the distribution of the IgG3 subclass in renal tissues was different for patients sensitive to immunosuppressive medications and those with refractory IMN, suggesting an important role for IgG3 in IMN's progression.

Based on the PLA2R gene polymorphisms, the GC genotype on the PLA2R SNPrs35771982 locus may be the risk factor for poor prognosis for IMN patients. In addition, patients with positive IgG3 in the renal tissues and a GC genotype on PLA2R SNPrs35771982 locus responded poorly to the immunosuppressive therapy and might need intensive treatment.

Significant differences existed in the ages, genders, BMIs, and IgG subclass distribution between the nonremission and remission groups. Based on this finding, clinicians should give immunosuppressive therapy to the patients with new-onset IMN according to current guidelines. Furthermore, the immunosuppressive therapy is less effective for middle-aged and senior male patients, patients with high BMI, and especially, patients with the positive IgG3 and the GC genotype on PLA2R SNPrs35771982 locus. The therapy needs use a larger dose in the early stage of IMN. Also, clinicians can increase the dose of ARB/ACEI for some patients, hoping for partial remission.

Overweight patients should decrease their body weight to the standard weight before treatment. Clinicians should replace one immunosuppressive medication with another one as early as possible in case of a poor effect of the first medication, aiming at realizing partial remission or complete remission.

Simultaneously, researchers need to explore the following two aspects further, namely whether patients with the GC genotype on the PLA2R SNPrs35771982 locus and a positive IgG3 show different responses to hormones combined with cyclophosphamide and rituximab, and whether scientists can customize the above two immunosuppressive medications according to pathological features and genotypes.

As for the patients with the refractory IMN and a negative conversion of the serum PLA2R antibody, clinicians need to observe their clinical outcomes for a longer time in the future, considering that the observation time may not long enough.

Refractory IMN is a common disease in the clinical practice, so researchers should summarize the clinical and pathological features and the gene polymorphisms of this disease continuously.

The current study had some limitations. The sample size was small and the observation time short. Hence, researchers need to perform studies in the future with larger sample sizes and observation times, thereby providing clinical evidence for individualized precision therapy.

CONCLUSIONS

The PLA2R gene polymorphism combined with the IgG subclass can predict the sensitivity of IMN patients to immunosuppressive therapy.

AUTHORS' DISCLOSURE STATEMENT

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest

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