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ORIGINAL RESEARCH

PLA2R1 Overexpression Causes Podocyte Injury by Inhibiting the Cell Cycle: A Clinical Cross-Sectional Investigation and Cellular Study

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Aim: Phospholipase A2 receptor 1 (PLA2R1) is often overexpressed in 70% of primary membranous nephropathy (PMN) patients, with serum PLA2R1 antibodies and podocyte PLA2R1 antigens serving as key diagnostic markers. However, a minority of patients test positive for only the PLA2R1 antigen and negative for PLA2R1 antibodies, presenting distinct characteristics. This study investigated the underlying features and mechanisms in PLA2R1 antigen-positive PMN patients.

Methods: 26 patients' information was screened for analysis. And the effects of PLA2R1 overexpression on human podocytes (HPCs) was studied through cell experiments.

Results: Clinical observations revealed that the median age of the 26 patients was 48.5 years, and the median onset time was 135 days. There was a significant negative correlation between blood albumin and antigen intensity. Cell studies demonstrated that PLA2R1 overexpression inhibited the proliferation and viability of HPCs. RNA sequencing and FACS assays revealed that PLA2R1 overexpression arrests HPCs at the S and G2/M phases.

Conclusion: PLA2R1 overexpression affects the course of the PMN by inhibiting the podocyte cycle. This study suggests that PLA2R1-related PMN pathogenesis could involve an additional immune response, offering insights into PMN treatment development.

Keywords: PLA2R1, membranous nephropathy, nephrotic syndrome, podocyte

Introduction

Membranous nephropathy (MN) is a unique glomerular disease and one of the most common adult nephrotic syndromes.[1] The pathogenesis of MN can be divided into primary membranous nephropathy (PMN) and secondary membranous nephropathy (SMN).[2,3] PMN is an autoimmune disease caused by the in situ production of immune complexes as a result of an autoantibody attack against antigens.[4] However, the exact pathogenesis is not fully understood. These patients account for approximately 80% of the total number of adults with MN.[5] SMN is secondary to well-defined causes, including infection, systemic disease, drug exposure, or malignancy, and is characterized by hematuria, proteinuria, edema, hypertension, and, in some cases, deep vein thrombosis and infection, which can lead to chronic kidney disease (CKD) or even serious chronic kidney failure (CKF).[6,7] In severe cases, hormone and immunosuppressive therapies can be used. Ultimately, patients who cannot be cured require kidney replacement therapy, such as dialysis or transplantation.[8] Early diagnosis and treatment are the keys to effectively preventing the development of kidney failure. Therefore, identifying the pathological mechanism of the PMN can aid early diagnosis and prognosis observation, which are important for early intervention and management.

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The criterion for confirming the diagnosis of MN is kidney puncture biopsy, but it does not apply to those who have a kidney biopsy contraindication or who are unwilling to undergo kidney biopsy themselves. The search for serum biomarkers of MN is certainly a safer option. In 2009, Beck et al performed protein immunoblotting experiments with serum and kidney tissue homogenates from adult MN patients and reported a 185 kDa protein in the kidney tissue of adult PMN patients who reacted with the patient's serum.[9] The reaction was not detected in the SMN, other glomerular diseases or healthy population. The band was later identified as phospholipase A2 receptor 1 (PLA2R1), which is highly expressed in the kidneys of PMN patients and expressed at low levels in the tissues of healthy individuals and patients with other kidney diseases. Further studies revealed that the antibody PLA2R1-IgG4, which reacts specifically with PLA2R1, was present in the serum of approximately 70% of PMN patients.[9] Statistics show that 85% of PMNs are affected by antibodies against PLA2R1, 3–5% are associated with THSD7A, and the pathological mechanism of the remaining 10% is not clear.[9–11] Therefore, patient serum PLA2R1 antibody levels are important criteria for the clinical diagnosis and prognosis of PMN.[12–15] However, some particular and uncommon patients whose serum PLA2R1 antibody is negative but whose kidney PLA2R1 antigen is positive have recently been reported. The onset mechanism of such patients has remained a mystery.

PLA2R is a member of the C-type lectin superfamily and a type I transmembrane receptor. PLA2R1, also known as M-type PLA2R, is the most studied PLA2R type and is expressed in several tissues in humans and animals. It can be upregulated by a variety of inflammatory factors and LPS stimuli.[16] PLA2R1 negatively regulates secretory phospholipase A2 (sPLA2), a function that is important for the tendency of PLA2R1 to suppress inflammation. However, the binding of sPLA2 to PLA2R1 contributes to the release of lipid mediators, thereby activating specific pathways for cell proliferation, migration, contraction, and secretion functions.[17,18] Moreover, PLA2R overexpression/activation-induced podocyte injury is associated with programmed cell death (PCD).[19–24] Furthermore, PLA2R1 is involved in regulating the senescence of normal cells and the death of cancer cells.[25,26] A study focused on prostate cancer revealed that the results of PLA2R1 overexpression were inconsistent between in vivo and in vitro settings.[27] In vivo experiments demonstrated that high expression of PLA2R1 increased the viability, proliferation, and migration ability of LNCaP-Ctrl and PC-3 KD cancer cells. In contrast, in vitro experiments revealed significant heterogeneity of PLA2R1 in different tumor cells. It promoted the growth of PC-3 cells but inhibited the growth of LNCaP and MDA-MB-453 cells. However, a study on kidney cancer indicated that decreased or knocked out PLA2R1 expression was related to tumorigenesis.[28] These studies suggest that PLA2R1 plays different roles in different cells. However, these findings could not explain the impact of PLA2R1 overexpression on podocytes.

This study hypothesized that overexpression of PLA2R1 in podocytes will lead to cell injury. This study aimed to explore the clinical characteristics of patients who were positive for only the podocyte PLA2R1 antigen via a cross-sectional clinical study. Second, on the basis of the results of the cell experiments, we investigated the influence and mechanism of PLA2R1 overexpression on human podocytes (HPCs). This study is important for understanding the pathogenesis and exploring future treatment methods for such patients.

Methods

Ethics

This study comprises two parts: a clinical cross-sectional study and a cell study. The clinical research mainly centers on data collation and statistics of clinical diagnoses and does not involve any intervention in patients. All patients signed the informed consent form. The relevant content was examined and approved by the Clinical Research Ethics Committee of Peking University Shenzhen Hospital (2022-123). HPCs are commercialized products (HUM-iCell-0110a, ICellBioscience, China). This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Patients and Testing

All patient information was obtained from those who were treated at our department from January 1, 2022, to December 31, 2023. The inclusion criterion was patients with supportive therapy in adult (age ≥ 18 yr), who were confirmed to be MN and positive for the PLA2R1 antigen via kidney biopsy. Exclusion criteria included: (1) patients

with positive serum PLA2R1 antibody detection; (2) those with systemic illnesses, malignancy, diabetes, or hepatitis B surface antigen positivity; and (3) individuals who had received prior steroids or immunosuppressive therapy. In addition, this study also excluded the expression of other PMN-related antigens to rule out the influence of multiple antigen expressions. All patients received concurrent testing for kidney tissue PLA2R1 antigen and serum PLA2R1-Ab. Consequently, all the obtained data were from patients who were positive for only the PLA2R1 kidney antigen.

Middle-aged and elderly patients with suspected MN typically exhibit the following clinical clues: patients at Age > 40 years (peak incidence 50–60 years), proteinuria, minimal or no red blood cells on urine microscopy, hypoalbuminemia (serum albumin < 3.0 g/dL), peripheral edema (eg, lower limbs, periorbital), hyperlipidemia; Serological Markers, positive anti-PLA2R antibodies. Kidney biopsy remains the gold standard for definitive diagnosis. The histopathological features indicative of PMN include: (1) Light microscopy: diffuse glomerular basement membrane (GBM) thickening; (2) Electron Microscopy: subepithelial electron-dense deposits; (3) Immunofluorescence: Predominant granular IgG4 deposits along the GBM. A definitive diagnosis of PMN requires fulfillment of all three criteria: clinical evidence of kidney injury, characteristic histopathological findings, and positive PLA2R1 antigen in kidney tissue and/or serum PLA2R1 antibody.

Kidney biopsies were performed at our department, and the relevant test reports were provided by the pathology department. The intensity of antigen positivity was judged by the same person in the pathology department. Blood antibody detection was carried out by Guangzhou Kingmed Diagnostics Group Co., Ltd. The biochemical indicators of patients were obtained through daily tests, as described previously.[29]

Bootstrap

To analyze the correlation between blood albumin (ALB), urine protein, and the categorical variable kidney antigen strength, the bootstrap method was employed. In the situation of a small sample size, the estimation results of traditional statistical methods may exhibit large deviations. Bootstrapping offers robust estimation results through repeated sampling and calculation, thereby reducing the statistical bias caused by small samples. Moreover, bootstrapping is a nonparametric method that does not rely on the distribution assumption of data and thus has better stability in this context. In this study, the bootstrap method estimates the Pearson correlation coefficient between variables by conducting 1000 resamplings.

Cell Culture and Treatment

HPC was cultured in a culture containing 1% Insulin-Transferrin-Selenium-A Supplement (ITS) at 33°C in a 5% CO2 incubator for proliferation. The medium was composed of 10% FBS (HyClone, USA) + 90% RPIM-1640 (Gibco, USA) + penicillin + streptomycin. The cells were passaged at 80% confluence. HPC was differentiated in a complete medium at 37°C and 5% CO2 for 7 days before the formal experiments. The cell culture medium was changed 3 times per week. Recombinant adenovirus carrying shRNA for PLA2R1 overexpression were designed by Han bio–Biotechnology Co Ltd (Shanghai, China). Adenovirus carrying a blank gene were used as control. After transfection for 72 h, the PLA2R1 protein expression level was detected via WB.

Immunofluorescence

The cells were transferred to glass-bottom culture dishes after 24 h of transfection and cultured for an additional 48 h. The cells were then incubated for 1 h with blocking solution (PBS+5% FBS+0.1 Triton-X 100) and then stained with the secondary antibody donkey anti-mouse IgG (H+L) secondary antibody Alexa Fluor 555 (A-31570, Thermo Fisher, USA) for 1 h at room temperature. The stained glass-bottom culture dishes were directly imaged with a Zeiss LSM 980 confocal microscope.

Cell Viability

Cell viability was assayed with a CCK-8 kit (C0037, Beyotime, China). Briefly, differentiated HPCs were precultured in 96-well plates (5000 cells/well) for 2 h before the experiment began. Cell viability assays were performed after 0 h (untreated). The assay procedure followed the manufacturer's instructions.[30] The cells at the end of the assay were not used for subsequent experiments.

RNA

The cells used for qRT–PCR detection were preserved in RNAwait (BL621A, Biosharp, China). For the cells used for RNA sequencing, after being lysed with TRIzol, they were directly sent to a third-party company (Ankejie, Guangzhou, China) for sequencing via express delivery with dry ice. For specific steps, refer to previous articles.[31,32]

FACS

Flow cytometry (FACS) was utilized to assess the impact of PLA2R1 overexpression on the HPC cell cycle. PLA2R1 was overexpressed via viral transfection when the HPC cell density reached 70–80%. After a 72-hour incubation period, the cells were harvested and stained using the Cell Cycle and Apoptosis Analysis Kit (C1052, Beyotime, China) as per the manufacturer's protocol. Subsequently, the fluorescence of $2*10^4$ cells per sample was measured using a CytoFLEX LX flow cytometer (Beckman Coulter, USA). The raw data were analyzed with FlowJo software (V10). This experiment was conducted four times independently to ensure reliability.

Western Blot (WB)

Protein extraction methods and assays were performed as described previously.[33] Briefly, the cells, protease inhibitor cocktail and RIPA lysate were mixed and lysed at 0°C for 30 min. Ultrasonic lysis was performed for 10 seconds. The protein concentration was detected with a BCA kit. The samples were mixed with SDS buffer and incubated at 95°C for 10 min. Protein expression was detected via WB via PLA2R1 (AMAB90775, SIGMA, USA), podocin (SAB4200810, SIGMA, USA), WT1 (A2446, ABclonal, China), α-Tubulin (RM2007, Beijing Ray Antibody Biotech, China), β-Actin (K200058M, Solarbio, China) and GAPDH antibodies (AC002, ABclonal, China). For cell identification, a protein ladder ranging from 10 to 180 was employed. For signaling pathway detection, a protein ladder spanning from 10 to 250 is utilized.

Statistics

In the clinical investigation, data are presented as the means ± standard deviations (SDs), median, or count (percentage), as appropriate. Pearson correlation coefficients was used to analyzed the correlations among the serum ALB concentration, urine protein concentration, and antigen strength, Bootstrap methods were used to compute due to the small sample size, which limits the robustness of traditional parametric methods. In the cell experiment, between-group comparisons were performed via Student's unpaired *t*-test. One-way ANOVA was used for comparisons of more than two groups, followed by post hoc tests when the ANOVA appeared significant. Differences were considered statistically significant when $p \leq 0.05$. Statistical analyses were performed with Python 3.11.9.

Results

Patient Information and Disease Characteristics

A total of 26 individuals were finally selected, including 14 males (53.85%) and 12 females (46.15%) (Table 1). The median age was 49.5 years for males, 42 years for females, and 48.5 years for all patients. The median onset time was 120 days for males, 150 days for females, and 135 days for all people. All enrolled patients had not received steroids or immunosuppressive drugs prior to kidney biopsy, however, a small proportion of patients (seven cases, 26.9%) had been administered renin-angiotensin system inhibitors (RASi) before the kidney biopsy procedure.

Table 1 Sample Demographics, Age, and Onset Time Statistics

	Count	Proportion (%)	Median Age (Years)	Median Onset Time (Days)
Male	14	53.85%	49.5	120.0
Female	12	46.15%	42	150.0
Overall	26	100%	48.5	135.0

Table 2 Categorization of Blood Albumin, Urine Protein, and Antigen Strength

Category	Levels	Count	Proportion (%)
Blood Albumin (g/L)	<30	10	38.46
	≥30 and <40	14	53.84
	≥40	2	7.69
Urine Protein (g/24h)	<1	6	23.07
	≥1 and <2	6	23.07
	≥2 and <3.5	6	23.07
	≥3.5	8	30.77
Renal Antigen Strength	1	7	26.92
	2	11	42.31
	3	8	30.77

Notes: Channelization of blood ALB, urine protein and antigen strength with proportions. Kidney antigen strength levels related to PlA2R1 are categorized as follows: Level 1 includes antigen strength levels of ±, ± - 1+, 1+; Level 2 includes 1+ - 2+, 2+; and Level 3 includes 2+ - 3+, 3+, 3+ - 4+.

We found that there were significant differences in the conditions of these patients. Therefore, a preliminary statistical analysis was performed (Table 2). There were 10 patients (38.46%) with a serum ALB concentration <30 g/L and 16 patients (61.54%) with a serum ALB concentration ≥30 g/L. Among them, only two patients (7.69%) had ≥40 g/L, which was normal. There were eight patients (30.77%) whose 24-hour urinary protein concentration was ≥3.5 g/L, which is within the range of nephrotic syndrome, and 18 patients (69.23%) whose protein concentration was <3.5 g/L, which is within the range of non-nephrotic syndrome proteinuria. There were 6 patients (23.07%) with a small amount of proteinuria, and the urinary protein concentration was <1 g/L. The immunofluorescence intensity of kidney PLA2R1 antigen can be divided into three grades: Level 1 includes antigen strength levels of ±, ± - 1+, and 1+; Level 2 includes 1+ - 2+, and 2+; and Level 3 includes 2+ - 3+, 3+, and 3+ - 4+. There were seven cases (26.92%) in Grade 1, 11 cases (42.31%) in Grade 2, and eight cases (30.77%) in Grade 3. A total of 18 cases (69.23%) presented medium and weak fluorescence intensities. The results of fluorescence detection of the kidney PLA2R1 antigen are shown in Figure 1A.

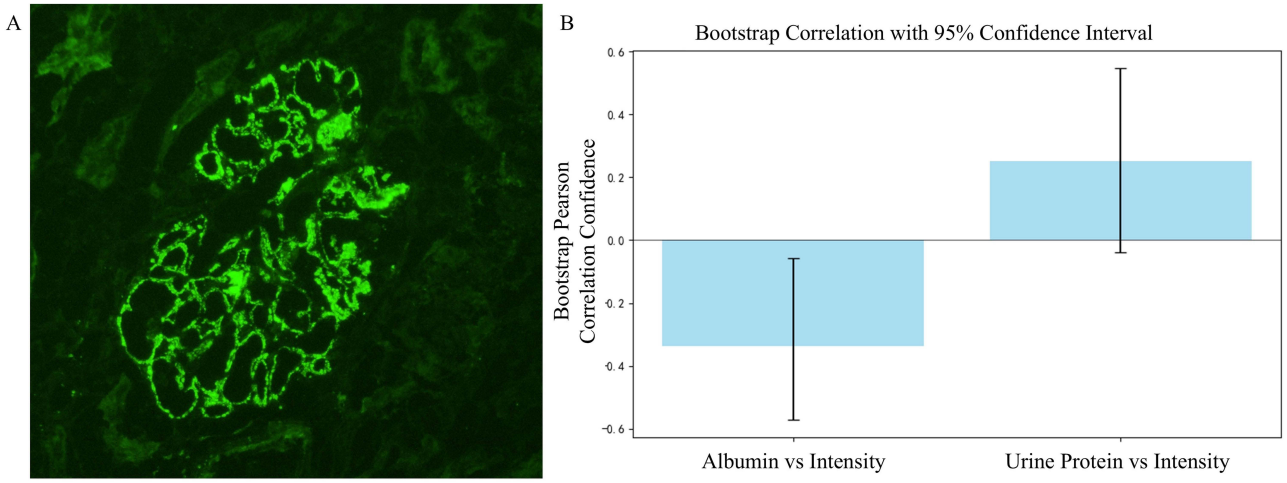

Figure 1 Information and disease-related characteristics of MN patients. (**A**) Representative immunofluorescence image of positive PLA2R1 antigen in the glomerular basement membrane of kidney biopsy samples. (**B**) Bootstrap correlation with 95% confidence interval. The blue color represents the bootstrap average correlation degree of blood ALB and urine protein with respect to intensity. The black interval indicates the 95% confidence interval of the correlation. If the black interval crosses the zero point, there is no significant correlation between the two variables.

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Next, we analyzed the correlations among the serum ALB concentration, urine protein concentration and antigen strength (Figure 1B). The bootstrap estimation revealed that the Pearson correlation coefficient between the serum ALB concentration and antigen strength was −0.34, with a 95% confidence interval of [−0.58, −0.05]. This result revealed that there was a significant negative correlation between the blood ALB concentration and antigen strength ($p \leq 0.05$), although the correlation was weak. Similarly, for urine protein and antigen strength, the Pearson correlation coefficient was 0.25, and the 95% confidence interval was [−0.05, 0.54]. Although the correlation coefficient is positive, suggesting a possible positive correlation between urine protein and antigen strength, considering that the confidence interval spans zero, it can be concluded that there is no significant correlation.

Identification and Transfection of HPC

First, the HPC was identified. The cells expressed the podocyte biomarkers podocin and WT1 (Figure 2A). Second, the transfection status was determined. We successfully prepared the plasmid and transfected it into HPCs via adenovirus. After transfection, PLA2R1 expression was examined. The study was divided into three groups: the original HPC group, the eGFP (enhanced green fluorescent protein)-HPC group, which only received eGFP transfection to confirm the transfection success rate; and the PLA2R1-HPC group, which received PLA2R1-eGFP transfection. WB results revealed that the original HPC and eGFP-HPC expressed a small amount of PLA2R1, whereas the expression level of PLA2R1 in PLA2R1-HPC significantly increased (Figure 2B). These findings indicate that the transfection was successful. Besides proteins, qRT-PCR also confirmed that the transfected HPCs highly expressed *Pla2r1* mRNA compared with the untransfected HPCs (Figure 2C).

To determine the distribution area of PLA2R1 protein, we examined it via microphotography. The photo showed that PLA2R1-monoclonal antibody (mAb) was uniformly attached to the surface of the PLA2R1-HPC membrane (Figure 2D). These findings indicate that PLA2R1 was successfully transfected in HPCs and that the PLA2R1 antigens are distributed on the surface of HPCs.

PLA2R1 Overexpression Inhibited HPCs Proliferation

Firstly, the cell density and morphology were observed to understand the effect of PLA2R1 on the cells. The transfection of the virus carrying eGFP significantly decreased the cell density and affected the cell morphology (Figure 3A and B). This indicates that transfection itself inhibits cell growth, but the overexpression of PLA2R1 further suppresses cell growth. Considering that an excessive number of variables can affect the result analysis, the subsequent experiments will only compare eGFP-HPC and PLA2R1-HPC.

To further determine the impact of PLA2R1 on cell morphology, the cells were stained using immunofluorescence. The results showed that PLA2R1 overexpression affected eGFP-HPC morphology and reduced cell size (Figure 3C and D). Additionally, cell viability is an important indicator of cell survival and proliferation. The CCK-8 results revealed no significant difference in cell viability between the groups after the initial 48 h of incubation. After 48 hours, PLA2R1 overexpression significantly reduced cell viability (Figure 3E). Given that 48 hours is ample time for HPCs to exit the stationary phase and commence proliferation, this finding implies that the reduced cell viability resulting from PLA2R1 overexpression may stem from diminished cell proliferation. However, the specific mechanism involved remains to be explored.

PLA2R1-Induced HPCs Injury Is Related to the Cell Cycle

To analyze the reasons why PLA2R1 inhibits the HPCs proliferation, RNA-seq was employed for detection. The results indicated that the overexpression of PLA2R1 led to significant changes in the expression levels of 631 genes, comprising 383 downregulated genes and 248 upregulated genes (Figure 4A and B). Moreover, the results of the comprehensive GO, KEGG, and REACTOME analyses suggested that PLA2R1 affects mainly the cell cycle, particularly the mitotic phase (Figure 4C–E). We identified the 23 genes with the greatest expression differences (Figure 4F). The accuracy of the RNA-seq results was verified by the sequencing company through qRT–PCR.

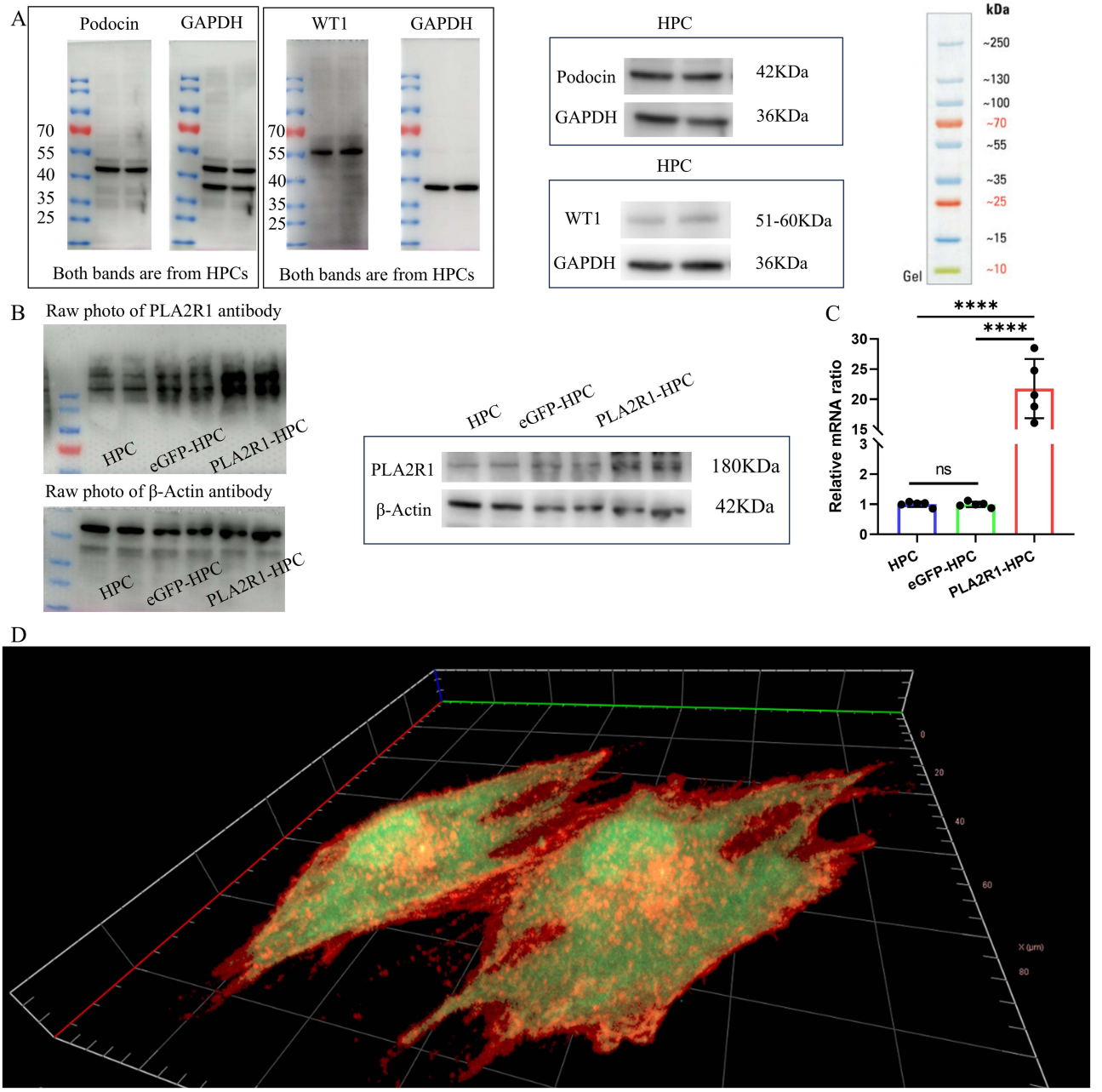

Figure 2 Identification and transfection of HPCs. (**A**) HPC can express podocin and WT1. This set of images includes the raw WB photos, the cropped target bands, and the protein ladder. Each sample occupies two wells. (**B**) Protein expression levels of PLA2R1 in original HPC, HPC transfected with eGFP (eGFP-HPC), and HPC transfected with PLA2R1 (PLA2R1-HPC). Protein expression results were measured 72 hours after transfection. This set of images includes the raw WB photos and the cropped target bands. Each sample occupies two wells. (**C**) The mRNA expression levels of *Pla2r1* in HPC, eGFP-HPC and PLA2R1-HPC. N=5. Students' *t*-test. ****$p \leq 0.0001$. (**D**) PLA2R1-mAbs bind to PLA2R1 expressed on the surface of the PLA2R1-HPC membrane. PLA2R1-mAb was labeled with red fluorescence. The cytoskeleton was labeled with green fluorescence.

PLA2R1 Overexpression Causes HPCs to Arrest at the S and G2/M Phases

To further validate the impact of PLA2R1 overexpression on the cell cycle and to identify the stage of cell cycle arrest, a cell cycle analysis was conducted. The findings revealed that overexpression of PLA2R1 decreases the percentage of HPCs in the G0/G1 phase while concurrently increasing the proportion of cells in the S and G2/M phases (Figure 5A and B). This suggests that HPCs are arrested during the S and G2/M phases of the cell cycle.

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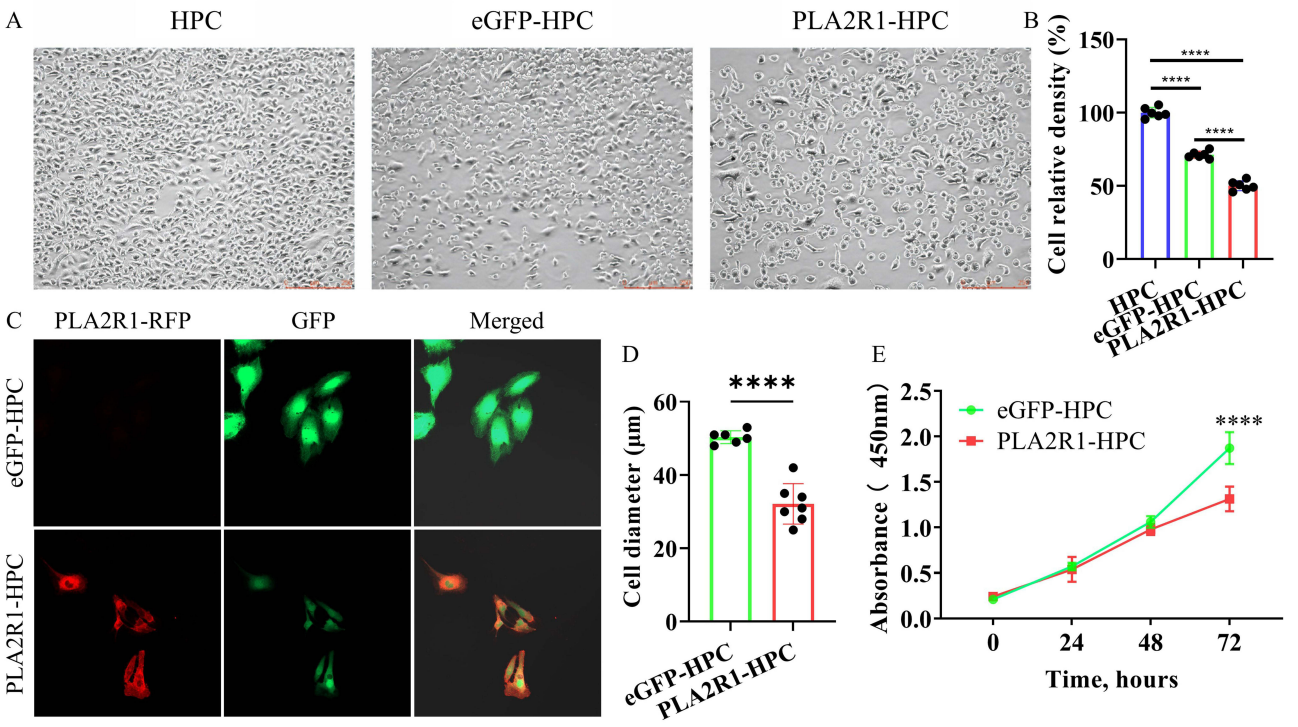

Figure 3 PLA2R1 overexpression inhibited HPC proliferation. (**A**) Representative photos of HPC, eGFP-HPC and PLA2R1-HPC. (**B**) Transfection inhibited cell growth, and PLA2R1 further suppressed it. N=5. (**C**) Representative images of cellular immunofluorescence staining. PLA2R1 was labeled on the membrane with a red fluorescent protein (RFP). The cells were labeled with green fluorescent protein (GFP). (**D**) PLA2R1 overexpression changed the cell morphology and diameter. N=5. (**E**) PLA2R1 overexpression inhibited cell viability. Students' *t*-test. ****$p<0.0001$.

Discussion

Approximately 70% of patients with PMN overexpress the antigen protein PLA2R1 in their kidneys, and the corresponding antibodies can be detected in the serum. The current mechanism is immune injury after antigen–antibody binding. However, we also observed that some patients diagnosed with PMN by pathology had positive PLA2R1 expression in the glomeruli but negative anti-PLA2R1 antibody detection in the serum, while they still exhibited obvious proteinuria or even nephrotic syndrome clinically. Although this group accounts for a small proportion of cases, its clinical characteristics and pathogenesis are not yet clear. To answer this question, we conducted preliminary research. Data from 186 patients who underwent kidney biopsy during hospitalization and were clinically diagnosed with PMN were collected from January 2022 to May 2024.

Among these patients, 26 had positive PLA2R1 expression in the glomeruli and negative anti-PLA2R1 antibody detection in the serum, accounting for 2.4% of all patients who underwent kidney biopsy at our hospital during the same period, and 14% had PMN. The median age was 48.5 years, and the median onset time was 135 days. Given that the sample size was relatively small, it was not possible to statistically analyze the sex differences. In clinical presentation, proteinuria >3.5 g per day and serum albumin <3.0 g/dl is present in around two-thirds of patients with PMN. But in our study, just one-thirds of patients who had positive PLA2R1 expression in the glomeruli and negative anti-PLA2R1 antibody detection in the serum presents proteinuria >3.5 g per day and serum albumin <3.0 g/dl. It appears that this small subset of patients has milder clinical manifestations. Although 26.9% of patients received RASi prior to kidney biopsy, which may potentially influence urinary protein quantification results, the following considerations support minimal confounding effects: (1) Compared with contemporaneous serum anti-PLA2R1 antibody-positive patients with PMN (RASi utilization rate 30%) at our center, the enrolled cohort demonstrated a lower RASi utilization rate; (2) The proportion of RASi-treated patients constituted a minor subgroup. We therefore conclude that pre-biopsy RASi exposure in this limited subpopulation would not substantially impact the study outcomes.

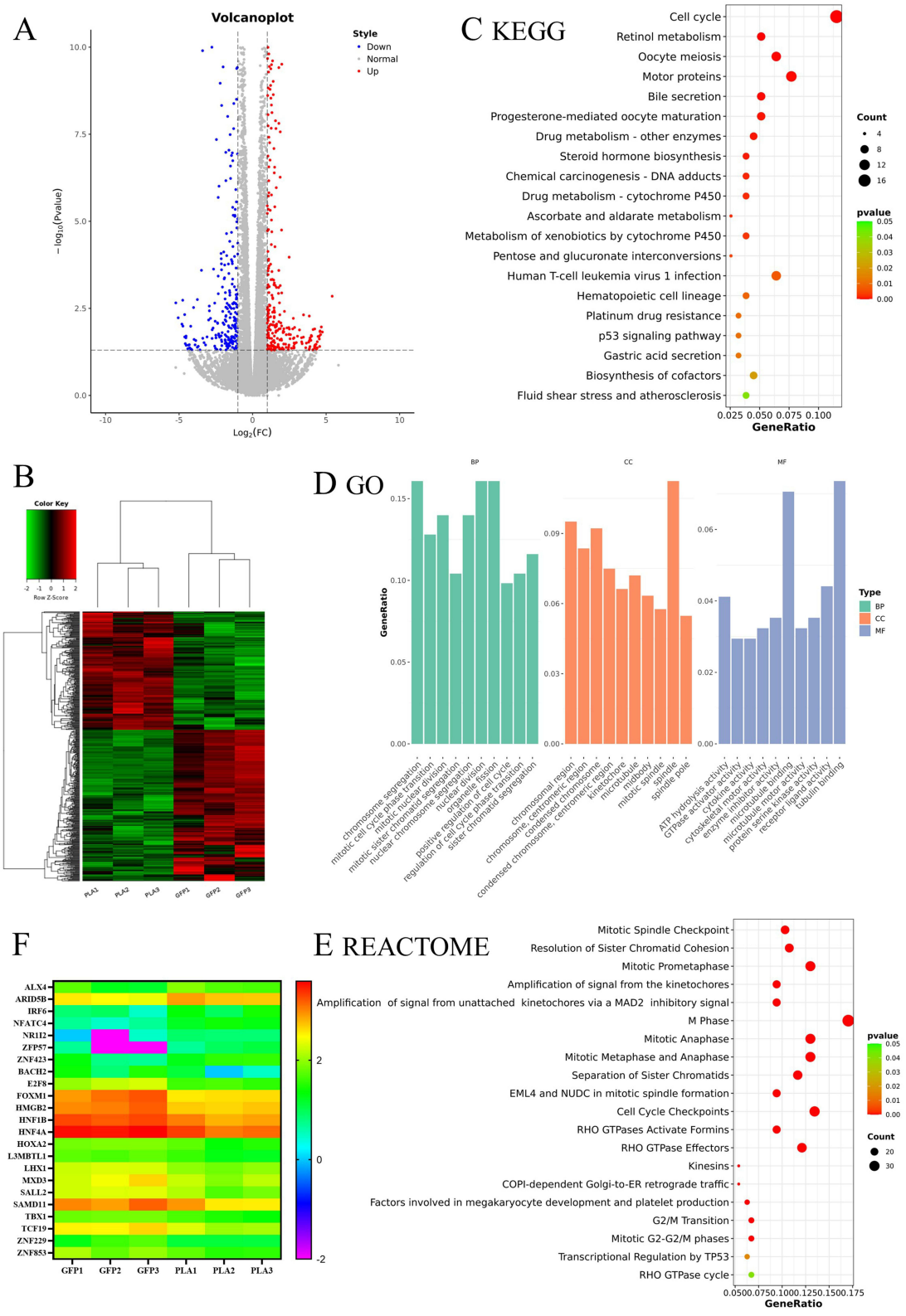

Figure 4 The RNA-seq results indicated significant differences in gene expression between eGFP-HPC and PLA2R1-HPC. N=3. (**A**) Volcano plot. (**B**) Heatmap of all genes whose expression significantly differed. (**C**) KEGG. (**D**) GO. (**E**) REACTOME. (**F**) Heatmap of the 23 genes with the most significant differences. The GFP1, GFP2 and GFP3 results are from eGFP-HPC. The PLA1, PLA2 and PLA3 results are from PLA2R1-HPC.

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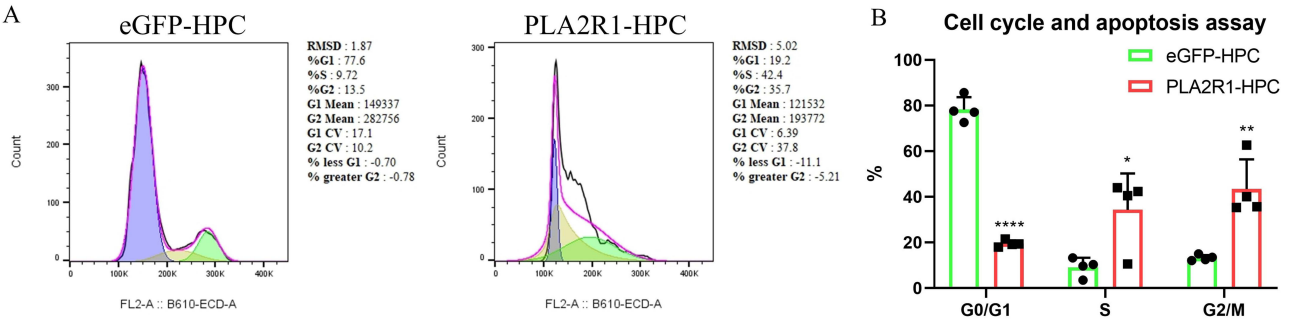

Figure 5 Overexpression of PLA2R1 causes HPCs to arrest at the S and G2/M phases. (**A**) Representative images of cell cycle detected by FACS. (**B**) Compared with eGFP-HPC, PLA2R1-HPC is mainly arrested at the S and G2/M phases. N=4. Students' *t*-test. *p<0.05. **p<0.01. ****p<0.0001.

Additionally, there is a negative correlation between the blood ALB level and antigen intensity. Cell studies further revealed the relevant mechanism involved. The overexpression of PLA2R1 inhibits cell cycle.

The most important finding of this study is that PLA2R1 overexpression does not directly lead to the death of HPCs but rather inhibits cell cycle. In the cell viability assay, we observed that the viability of the eGFP-HPC consistently increased. However, the activity of PLA2R1-HPC is consistent with that of the former within 48 hours (Figure 3E). It was not until after 48 hours that the rate of increase in viability decreased. The decrease in cell viability is usually associated with the reduction in cell proliferation ability and the increase in cell death. However, no significant cell death was observed morphologically (Figure 3A and C). This suggests that the reduction of cell viability induced by PLA2R1 might be associated with the decline of cell proliferation. Subsequent results of RNA sequencing and FACS assays showed that the overexpression of PLA2R1 inhibited cell cycle-related signals (Figures 4 and 5). In other words, the overexpression of PLA2R1 suppressed cell proliferation. Generally, podocyte injury is considered irreversible.[34] However, recent studies have demonstrated that podocytes can proliferate and repair under specific conditions.[35] Integrating these findings with this study results, we propose that PLA2R1 overexpression may disrupt cellular homeostasis by perturbing the cell cycle, thereby impairing podocyte function and diminishing their capacity for repair.

These findings indicate that abnormal immune function may be an important reason for podocyte injury in clinical practice. For example, Reinhard et al reported that injecting PLA2R1 antibodies from MN patients into pigs could cause MN.[36] Therefore, the overexpression of the PLA2R1 antigen may have accelerated the disease process by reducing cell activity. Additionally, some studies have suggested that both the overexpression and reduction of PLA2R1 can lead to cell cycle defects.[28,37] These findings also suggest that stable expression of PLA2R1 is crucial for the cell cycle and cell activity and that damage to podocytes may require the participation of exogenous factors.

Moreover, appropriate expression of PLA2R1 is important for HPCs, as both HPCs and eGFP-HPCs expressed a small amount of PLA2R1. A recent study revealed that PLA2R1 deficiency can also cause CKD symptoms in rats, such as persistent proteinuria, decreased podocyte count, and increased expression of complement C3.[38] These findings suggest that normal expression of PLA2R1 is important for the health of podocytes. Both overexpression and underexpression can disrupt cellular homeostasis.

Currently, PMN treatment is based on supportive therapy, and severe patients can be treated with immunosuppressive drugs or monoclonal antibody drugs; however, full recovery is related to the patient's self-healing ability. For example, Rituximab induces B lymphocyte apoptosis by binding to CD20, thereby reducing anti-PLA2R1 antibody generation. However, the recovery of some patients is unsatisfactory. This might be because the regenerative capacity of podocytes is very weak. Moreover, the overexpression of PLA2R1 further diminishes its environmental adaptability and repair ability. As a result, the glomeruli of these patients are more likely to be damaged and difficult to repair. In future treatments, it may be necessary not only to reduce PLA2R1 antibodies in the blood but also to consider targeting the PLA2R1 antigen on podocytes.

There are limitations in this study. First, no animal experiments were conducted. Second, potential mechanisms of the decrease in cell viability still need to be explored. Third, we did not compare the disparities between patients with single

PLA2R1 antigen positivity and other patients with PMN. This is mainly due to the scarcity of such patients, which leads to the difficulty in collecting relevant data. This is also a project currently in progress. Finally, we did not resolve the protein structure. In the future, the protein structure will be resolved by cryo-electron microscopy, which will be useful for developing novel drugs targeting PLA2R1. All these directions are worth exploring in depth in the future.

Conclusion

In conclusion, the overexpression of PLA2R1 may impact podocyte health by inhibiting the cell cycle. Thus, the PLA2R1 antigen may also be an important target for the treatment of PMN. This study provides a new perspective for understanding the pathological mechanism of PMN and developing new treatment strategies.

Abbreviations

ALB, albumin; BAX, *Bcl2*-associated X protein; BCL-2, B-cell lymphoma 2; CCK-8, Cell Counting Kit-8; CKD, chronic kidney disease; CKF, chronic kidney failure; eGFP, enhanced green fluorescent protein; HPC, human podocyte cell line; mAb, monoclonal antibody; PLA2R1, phospholipase A2 receptor 1; MN, membranous nephropathy; PMN, primary membranous nephropathy; PCD, programmed cell death; RASi, renin-angiotensin system inhibitors; SMN, secondary membranous nephropathy; sPLA2, secretory phospholipase A2; WB, Western Blot.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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