

Association between Nod-like receptor protein 3 inflammasome and gouty nephropathy

YAN-ZI ZHANG, XIAO-LU SUI, YUN-PENG XU, FENG-JUAN GU, AI-SHA ZHANG and JI-HONG CHEN

Department of Nephrology, Affiliated Baoan Hospital of Shenzhen, The Second School of Clinical Medicine, Southern Medical University, Shenzhen, Guangdong 518000, P.R. China

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Abstract. Crystallized deposits of monosodium urate activate the Nod-like receptor protein 3 (NLRP₃) inflammasome, resulting in kidney damage. The present study investigated whether the NLRP₃ inflammasome is associated with the progression of hyperuricaemia and gouty nephropathy. Adult male patients were recruited at the Affiliated Baoan Hospital of Shenzhen and divided into three groups of 15 patients each: The control group, the hyperuricaemia group and the gouty nephropathy group. General characteristics and organ function indicators were also measured for each patient. NLRP₃, apoptosis-associated speck like protein (ASC) and caspase-1 mRNA and protein expressions in peripheral blood mononuclear cells were detected. The expression of certain downstream inflammatory factors, including interleukin (IL)-1 β and IL-18 were also assessed in plasma. The results demonstrated that the concentration of uric acid and creatinine were increased in the hyperuricaemia and gouty nephropathy groups compared with the control group. NLRP₃, ASC and caspase-1 mRNA and protein expression, and IL-1 β and IL-18 expression were increased in the hyperuricaemia and gouty nephropathy groups compared with the control group. In addition, ASC and caspase-1 mRNA and protein expression, and IL-1 β expression were higher in the gouty nephropathy group compared with the hyperuricaemia group. In conclusion, the present results supported the hypothesis that the NLRP₃ inflammasome signalling pathway is associated with gouty nephropathy leading to initiation of the inflammatory response and causing renal damage.

Introduction

The role of uric acid in the causation and progression of chronic kidney disease (CKD) has been debated; however,

uric acid has been reconsidered as a potential contributory risk factor for the development and progression of CKD over the last 15 years (1-2). Excessive uric acid, usually in the form of monosodium urate (MSU) crystals, precipitates in synovial cavities and other anatomic location to induce severe inflammation and debilitating pain (3-6). In particular, deposits of uric acid in the kidney causes gouty nephropathy, which is the most serious complication of hyperuricemia (3-6). It is considered that the activation of renin-angiotensin, inflammatory factors, endothelial dysfunction and cyclooxygenase-2 (COX-2) serve important roles in gouty nephropathy (5,6). The identified underlying mechanisms of gouty nephropathy have revealed that inflammation has a dominant role in its pathogenesis.

The Nod-like receptor protein 3 (NLRP₃) inflammasome is comprised of NLRP₃, apoptosis-associated speck like protein (ASC) and caspase-1 and is the most extensively studied inflammasome of recent years. It is involved in certain human inflammatory and autoimmune diseases, including cryopyrin-associated periodic syndrome, ischaemia reperfusion injury and atherosclerosis (7,8). Following detection of cellular stress, NLRP₃ oligomerizes by homotypic interactions between NACHT domains. The pyrin domains (PYD) of NLRP₃ then becomes exposed for ASC binding. The caspase activation and recruitment domains (CARD) of ASC in turn recruits pro-caspase-1 through CARD-CARD interactions (9). Following NLRP₃ inflammasome formation, procaspase-1 is converted to active caspase-1, which regulates the maturation of proinflammatory cytokines, including interleukin (IL)-1 β and IL-18 (10), further aggravating renal damage.

MSU crystals cause the formation of inflammasomes *in vivo* (11). In addition, the inflammatory effect of MSU crystals is primarily mediated by NLRP₃ inflammasomes driving the production of IL-1 β and IL-18. IL-1 β is likely the main agent that triggers systemic inflammation (3). Therefore, these observations prompted the present study to assess the role of the NLRP₃ inflammasome in the mediation of the innate immune inflammatory response to MSU crystal deposition with regards to gouty nephropathy. The present study investigated the role of the NLRP₃ inflammasome signalling pathway with the progression of hyperuricemia and gouty nephropathy, the results of which may provide a novel theoretical basis and therapeutic target for the early prevention and treatment of gouty nephropathy.

Correspondence to: Dr Ji-Hong Chen, Department of Nephrology, Affiliated Baoan Hospital of Shenzhen, The Second School of Clinical Medicine, Southern Medical University, 118 Longjing Road, Xinan Street, Baoan, Shenzhen, Guangdong 518000, P.R. China
E-mail: chenjihong0606@hotmail.com

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Materials and methods

Study subjects. A total of 45 male patients (18-70 years old) were recruited at the People's Hospital of Shenzhen Bao'an between July 2016 and December 2017. According to the inclusion and exclusion criteria, these patients were divided into three groups (n=15): The control group, the hyperuricaemia group and the gouty nephropathy group. The present study was approved by the Ethics Committee of the Affiliated Bao'an Hospital of Shenzhen (approval no. BYL2016001). Written informed consent was obtained from all participants.

Inclusion criteria. Patients in the control group received a health examination. There were no abnormalities in the laboratory indicators of the selected subjects and patients had no history of cardiovascular disease or liver disease (including diabetes and gout). Patients also had no presence of infection or autoimmune disease. Hyperuricaemia was defined as levels of serum uric acid >6-7 mg/dl (12). The diagnosis of gouty nephropathy was based on the diagnosis of primary gout (13), with one or more of the following parameters: Urinary protein >150 mg/dl; urine white blood cells >5/high power field (HPF); urine red blood cells >3/high power field; serum creatinine >115 μ mol/l; blood uric acid/creatinine ratio >2.5; ultrasound or ureterography revealing renal calculus and kidney shrinkage. All of the aforementioned cases excluded urinary tract infections and other diseases such as cancer.

Exclusion criteria. Exclusion criteria was based on previous literature (14) and was as follows: female; <18 years old or >70 years old; patients with secondary hyperuricaemia or stage 4-5 chronic kidney disease; acute hyperuricaemia and the presence of acute renal function deterioration factors; patients with severe cardiovascular disease, liver and kidney disease, lung disease, fractures, tumors, infectious and autoimmune disease, and mental illness; diseases that may affect NLRP₃ inflammasome signalling pathways; patients who had been using uric acid drugs outside the hospital or had been treated with lipid-lowering drugs or anti-inflammatory and anti-oxidative drugs during the 4 weeks prior to admission.

Detection of organ function indicators. Biochemical serum and urine samples were obtained following 8 h fasting. A total of 15 ml serum sample was collected from each patient and shipped to the Laboratory Services at the Affiliated Bao'an Hospital of Shenzhen (Guangdong, China) for biochemical analysis, which was obtained by centrifugation at 500 x g for 10 min at 4°C. Urinary biochemical parameters were measured from the patients' first morning urine sample. Other standard parameters were measured including blood and urine analysis. The parameters tested for blood included white blood cell (WBC), red blood cell (RBC), hemoglobin (HB), platelets (PLT), total cholesterol (TC), total triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine (Cr), uric acid (UC), blood urea nitrogen (BUN), prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) and fibrinogen (FIB). Other parameters tested for urine were urine leukocytes, urine erythrocytes and urine protein.

Isolation and culture of peripheral blood mononuclear cells. Centrifugation (speed, 1,600 x g; duration, 10 min; temperature, 4°C) was performed on blood samples in an EDTA tube to obtain the buffy coat. Samples were stored at -80°C for future use. Peripheral blood mononuclear cells were isolated with Ficoll-Paque Plus (GE Healthcare). Monocytes were isolated by magnetic bead negative selection according to the instructions of Dynabeads® Untouched™ Human Monocytes kit (Invitrogen; Thermo Fisher Scientific, Inc.).

Isolated monocyte lysates were separated from which 5 μ l was extracted for viability verification. Trypan Blue staining confirmed that the viability of the cells was >95%. Cell concentration was then adjusted to 5x10⁵ cells/ml using a hemocytometer using the following formula: Number of cells/ml = number of cells counted in 100 small grids/100x400x10,000x dilution factor. The total number of monocytes was counted to be 34.311±19.912x10⁵. A third of which was used for RNA extraction, whilst the rest were used for western blotting. The number of monocytes for RNA extraction and western blot analysis were divided 1:2. A total of 200 μ l TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.) was added to monocytes for RNA extraction, whereas 30-50 μ l PBS (Invitrogen; Thermo Fisher Scientific, Inc.) was added for western blotting experiments.

Reverse transcription-quantitative PCR (RT-qPCR) to determine NLRP₃, ASC and caspase-1 mRNA expression in peripheral blood mononuclear cells. Total RNA was extracted from monocytes using TRIzol solution (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. RNA concentration and purity were assessed via electrophoresis and a UV spectrophotometer (Thermo Fisher Scientific, Inc.), respectively. RNA was reverse transcribed to cDNA following the protocol provided by the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.). cDNA was stored at -20°C prior to qPCR amplification using the TransStart® Tip Green qPCR Supermix 2X kit (TransGen Biotech Co., Ltd.), using the SYBR-Green I fluorophore, according to manufacturer's protocol. All qPCR reactions were performed in an Applied Biosystems 7500 system (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the following thermocycling conditions: Initial denaturation at 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec and 60°C for 30 sec. Finally, the melting curve was generated (dissociation, 60°C at 30 sec and 95°C at 15 sec) to determine normality. Gene expression was calculated using the 2^{- $\Delta\Delta C_q$} method (15). β -actin was used as an internal reference for the mRNA levels of NLRP₃, ASC and caspase-1 detected. The primer sequences used for qPCR are presented in Table I.

Western blot analysis to determine NLRP₃, ASC and caspase-1 protein expression in peripheral blood mononuclear cells. Cells were lysed using ice-cold RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd.) and protein concentration was quantified using Bicinchoninic Acid Assay kit (Thermo Fisher Scientific, Inc.). Protein samples (10 μ g) were separated by 8, 10 or 12% SDS-PAGE before being transferred to PVDF membranes. The membranes were then blocked with 5% milk dissolved in TBS supplemented with 1% Tween-20 at room temperature for 1 h, prior to incubation with

Table I. Primer sequences.

Gene	Forward primer	Reverse primer
β -actin	5'-AACCGCGAGAAGATGACCCAGAT-3'	5'-GGATAGCACAGCCTGGATAGCA-3'
NLRP ₃	5'-ATGGGTTTACTGGAGTACCTTTC-3'	5'-CTGTCTTCAATGCACTGGAATCTG-3'
ASC	5'-GATGCTCTGTACGGGAAGGTC-3'	5'-TCCAGTTCCAGGCTGGTGT-3'
Caspase-1	5'-GGAAGACTCATTGAACATATGCAAG-3'	5'-CTTGTCAAAGTCACTCTTTCAGTG-3'

NLRP₃, nucleotide-binding domain and leucine-rich repeat protein 3; ASC, apoptosis-associated speck like protein.

Table II. General characteristics of enrolled patients.

Characteristics	Control (n=15)	Hyperuricemia (n=15)	Gouty nephropathy (n=15)
Age (years)	45.20±10.19	48.27±14.91	48.3±11.5
Height (m)	1.72±0.06	1.70±0.05	1.66±0.05
Body weight (kg)	72.25±6.97	69.98±12.08	61.39±6.83
BMI (kg/m ²)	24.28±1.53	24.38±4.53	22.19±2.43
SBP (mmHg)	116.00±7.61	115.93±9.83	121.33±10.87
DBP (mmHg)	79.27±7.98	76.47±7.60	74.80±8.78
FBG (mmol/l)	4.73±0.45	5.17±0.70	4.77±0.39

Values are presented as the mean ± standard deviation. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic pressure; FBG, fasting blood-glucose.

primary antibodies against NLRP (1:300; cat. no. sc-134306; Santa Cruz Biotechnology, Inc.), caspase-1 (1:300; cat. no. sc-622; Santa Cruz Biotechnology, Inc.), ASC (1:300; cat. no. sc-514414; Santa Cruz Biotechnology, Inc.) or GAPDH (1:1,000; cat. no. A01020; Abbkine, Inc.) diluted in PBS supplemented with 0.1% Tween-20 (PBST) and 1% bovine serum albumin [Sangon Biotech (Shanghai) Co., Ltd.] at 4°C overnight. Subsequently, the PVDF membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse (1:1,000; cat.no. A0216; Beyotime Institute of Biotechnology) or goat anti-rabbit (1:10,000; cat. no. A21020-1; Abbkine, Inc.) secondary antibodies diluted in PBST supplemented with 5% milk at room temperature for 1 h with agitation. The proteins were then visualized using Pierce™ ECL Western Blotting Substrate (Thermo Fisher Scientific, Inc.) and all bands were analysed using Gel Doc™ XR+ Gel Documentation system (Bio-Rad Laboratories, Inc.) to calculate the densitometric values.

ELISA for the determination of IL-1 β and IL-18 levels in plasma. IL-1 β (cat. no. 70-EK101B2) and IL-18 (cat. no. 70-EK1182) ELISA kits [Hangzhou Multi Sciences (Lianke) Biotech Co., Ltd.] were used to determine the concentration of IL-1 β and IL-18 in 2 ml total plasma, according to manufacturer's protocol.

Statistical analysis. Each experiment was repeated three times. All data were analysed using SPSS v.17.0 (SPSS, Inc.) software and expressed as the mean ± standard deviation.

One-way analysis of variance was performed to compare the mean of multiple groups and a least significant difference post hoc test was used. P<0.05 was considered to indicate a statistically significant difference.

Results

Kidney function is damaged in hyperuricaemia and gouty nephropathy groups. Patient general characteristics are provided in Table II, Figs. 1 and 2. The expression of uric acid (Fig. 1C), blood urea nitrogen (Fig. 1B) and blood lipids (Fig. 1F) including total cholesterol, total triglycerides and low-density lipoprotein was higher in the hyperuricaemia and gouty nephropathy groups compared with the control group, but the level of high-density lipoprotein exhibited the opposite trend. Levels of urine leukocytes and erythrocytes were higher in the gouty nephropathy group compared with both the control and hyperuricemia groups (Fig. 2E-F). Serum creatinine (Fig. 1A), uric acid (Fig. 1C), blood urea nitrogen (Fig. 1B) were greater in the gouty nephropathy group and hyperuricaemia group compared with the control group. Serum creatinine and urine protein are common indicators of renal function and used to monitor the progression of chronic kidney disease (16). These results demonstrated that the kidney had been further damaged in the progression from hyperuricaemia to gouty nephropathy.

Successful peripheral blood mononuclear cell isolation. Peripheral blood mononuclear cells were isolated from the

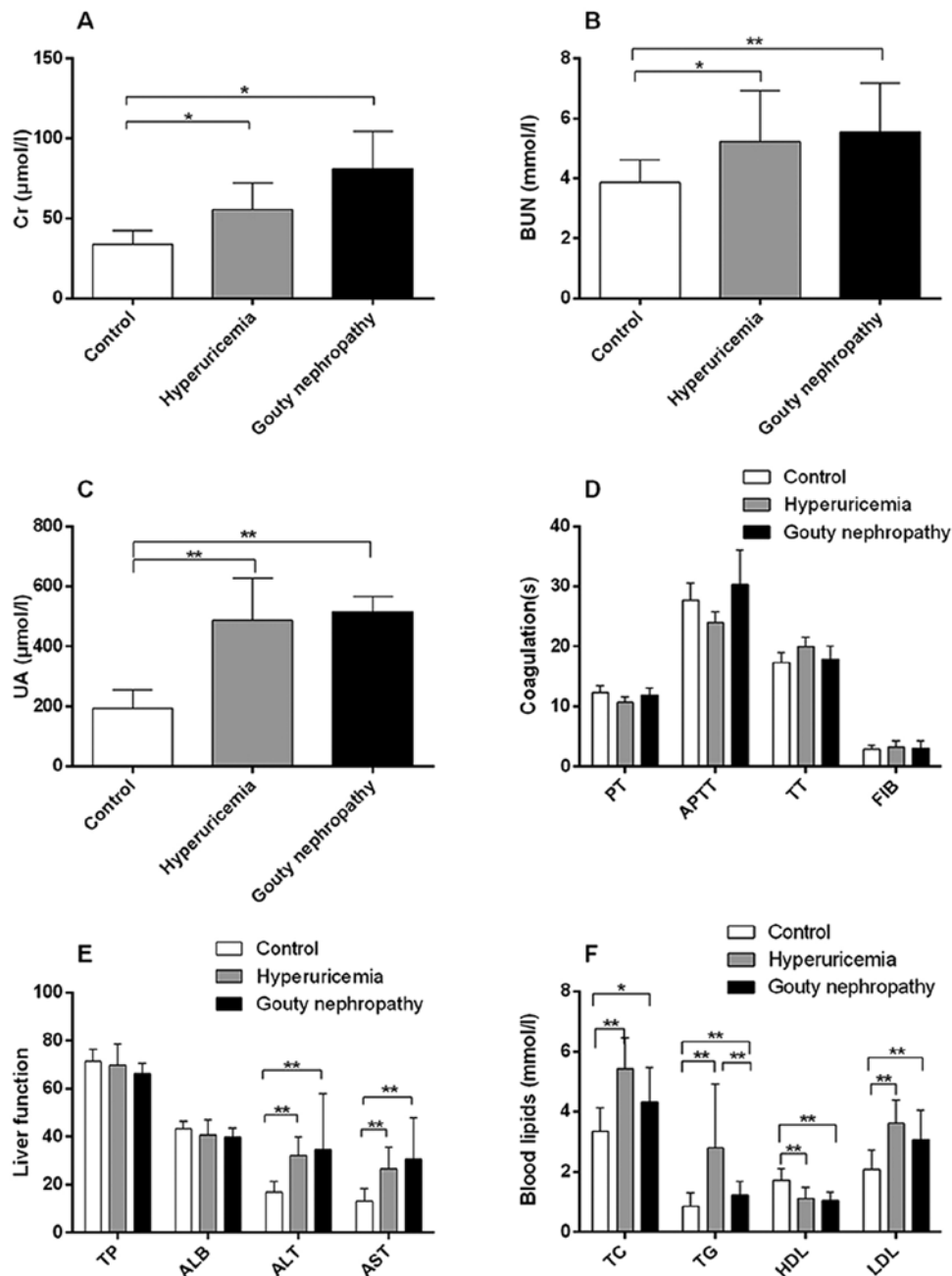


Figure 1. Organ function indicators of enrolled patients, including renal and liver function, coagulation and blood lipids. (A) Levels of Cr, (B) BUN, (C) UA, (D) coagulation functions, (E) liver function and (F) blood lipids of patients in the control, hyperuricemia and gouty nephropathy groups. * $P < 0.05$ and ** $P < 0.01$, with comparisons indicated by lines. Cr, creatinine; BUN, blood urea nitrogen; UA, uric acid; PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen; TP, total protein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; TG, total triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein.

peripheral blood of 45 patients for *in vitro* experiments. Trypan blue staining (Fig. 3) confirmed that >95% were viable cells (Table III).

NLRP₃, ASC and caspase-1 mRNA expression are increased in the hyperuricaemia and gouty nephropathy groups. NLRP₃, ASC and caspase-1 mRNA levels were significantly increased in the hyperuricaemia and gouty nephropathy groups compared with the control group (Table IV). Compared with the hyperuricaemia group, the gouty nephropathy group had a significantly higher expression of ASC and caspase-1 mRNA (Table IV). The mRNA expression in peripheral blood

mononuclear cells demonstrated that the NLRP₃ inflammasome may serve a pivotal role in gouty nephropathy.

Expression of NLRP₃, ASC, caspase-1 protein increases in the hyperuricaemia and gouty nephropathy group. Gouty nephropathy group had a significantly higher expression of NLRP₃, ASC and caspase-1 protein compared with the control group (Table V). The expression of ASC and caspase-1 protein was higher in the gouty nephropathy group compared with the hyperuricaemia group (Table V; Fig. 4). Peripheral blood mononuclear cell protein expression (Fig. 4; Table V) also corresponded with mRNA expression (Table IV), indicating

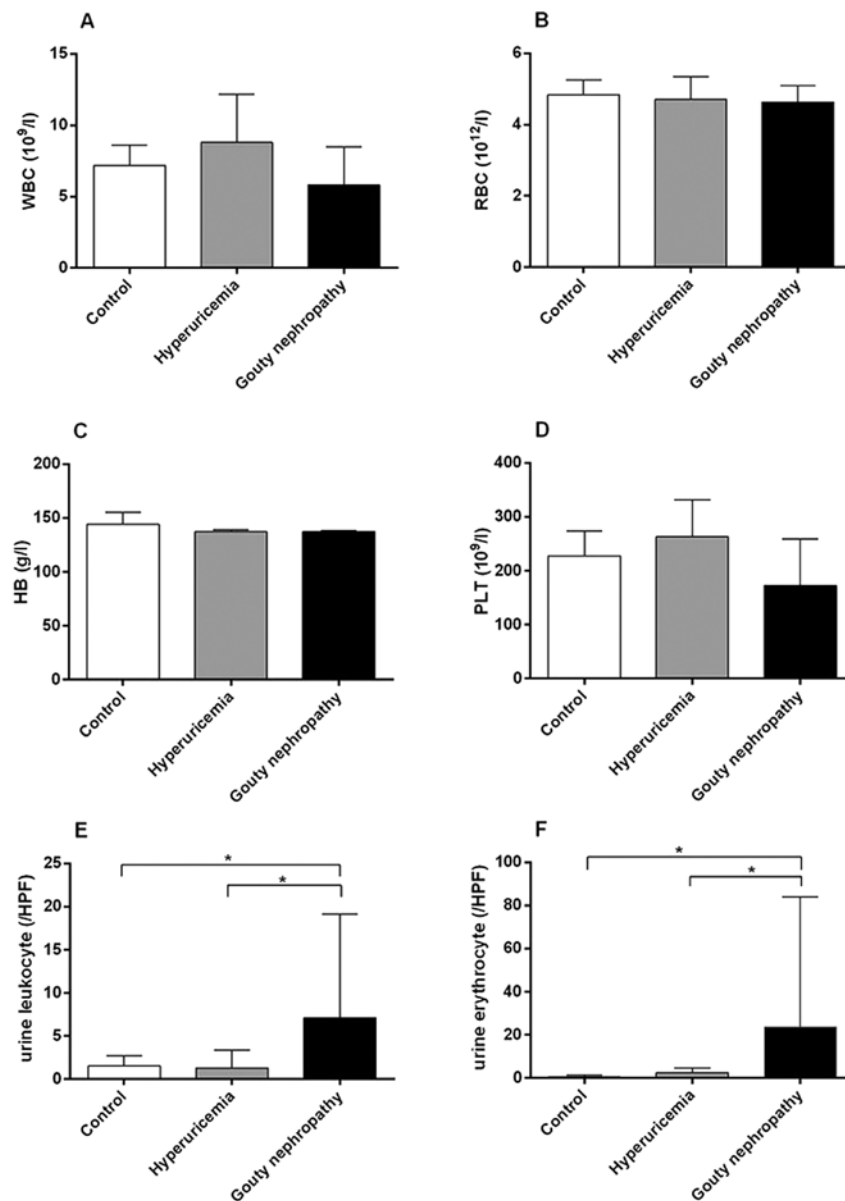


Figure 2. Organ function indicators of enrolled patients, including blood and urine analysis. (A) WBC, (B) RBC, (C) HB, (D) PLT, (E) urine leukocytes and (F) urine erythrocytes levels of patients in the control, hyperuricemia and gouty nephropathy groups. * $P<0.05$, with comparisons indicated by lines. WBC, white blood cell; RBC, red blood cell; HB, hemoglobin; PLT, platelets; HPF, high power field.

the important role of the NLRP₃ inflammasome in gouty nephropathy.

Expression of IL-1 β and IL-18 is increased in the hyperuricaemia and gouty nephropathy group. IL-1 β and IL-18 levels were significantly higher in the hyperuricaemia and gouty nephropathy groups compared with the control group (Table VI). IL-1 β expression was significantly elevated in the gouty nephropathy group compared with the hyperuricaemia group (Table VI). The increased expression of inflammatory factors demonstrated that the NLRP₃ inflammasome was associated with gouty nephropathy.

Discussion

Uric acid is involved in the pathogenesis of kidney disease. Increasing evidence supports uric acid as a cause or

exacerbating factor for kidney fibrosis and progressive CKD with an elevated serum uric acid level independently predicting the development of CKD (17,18). Elevated uric acid levels induce oxidative stress and endothelial dysfunction, resulting in the development of both systemic and glomerular hypertension (19,20). It is well established that uric acid forms urate crystals in sufficient concentrations to block renal collecting tubes, which causes interstitial nephritis, gouty nephropathy and interstitial fibrosis (21). Furthermore, the kidney damage caused by uric acid is the result of this sort of obstruction and crucially, it can be exacerbated by the inflammatory response that it initiates. Uric acid activates cytoplasmic phospholipase A2 and inflammatory transcription factor NF- κ B, which increases the production of various systemic cytokines, including tumour necrosis factor- α , kidney monocyte chemoattractant protein 1 and blood vessel COX-2 (22,23). In addition, hyperuricaemia aggravates kidney injury by

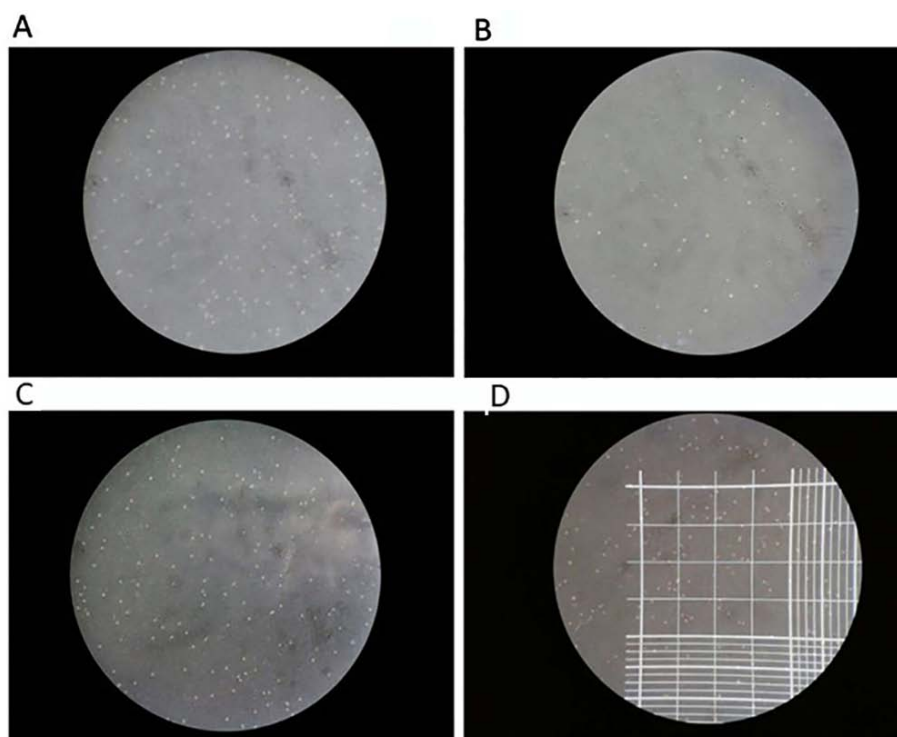


Figure 3. Representative images of trypan blue staining of peripheral blood mononuclear cells (magnification, x10). Trypan Blue staining test was used to confirmed the cell viability, followed by the counting of cells. Trypan blue staining can stain dead cells blue, whilst the live cells are not colored. (A) Densely distributed colorless transparent particles under the microscope, representing live cells. (B) Scattered distribution of colorless transparent particles, showing fewer living cells. (C) Densely distributed colorless transparent particles, representing live cells. (D) Cell counting schematic. The area of every grid is 1.0 mm² and the volume is 0.1 μ l. Cells in the double-lined area were counted as red blood cells, whereas cells in the single-lined area counted as white blood cells. Magnification, x10.

Table III. Peripheral blood mononuclear cells count.

Group	n	Count (10 ⁵)
Control	15	33.047±22.755
Hyperuricemia	15	43.627±18.317
Gouty nephropathy	15	26.567±13.506

recruiting IL-1 β -secreting macrophages, activating NLRP₃ inflammasomes in macrophages and promoting chemokine secretion in proximal tubular cells (24). Uric acid, in its soluble form, is responsible for increasing the production of IL-1 β in an NLRP₃-dependent manner and is associated with kidney damage (25).

The inflammatory response is closely associated with the innate immune system. Intracellular nucleotide-binding oligomerization domain-like receptors form a group of pattern recognition receptors that are involved in a wide variety of innate host immune responses when stimulated by pathogen-associated molecular patterns dangerous-associated molecular patterns (DAMPs) (26). One of the most thoroughly investigated members is the intracellular pattern recognition receptor NLRP₃ inflammasome complex consisting of NLRP₃, ASC and caspase-1 (27). Upon activation, NLRP₃ oligomerizes via homotypic interactions between NACHT domains and raises ASC and caspase to form an inflammatory complex, resulting in activated caspase-1. Activated caspase-1 cleaves intracellular IL-1 β and IL-18 precursors to form mature

IL-1 β and IL-18, which are secreted into the extracellular matrix (Fig. 5) (28). The inflammasome is understood to have a fundamental role in the progression of autoinflammatory diseases and to have additional roles in infection control, the progression of immune pathologies and the recognition of tissue damage. NLRP₃ inflammasome is activated in response to a variety of infectious stimuli or by cellular stress caused by various sterile danger signals, including high concentrations of extracellular ATP, a decrease in extracellular osmolality or pH, crystals of MSU or cholesterol and the degradation of extracellular matrix components (27).

In the present study, the concentration of uric acid and creatinine as well as the expression of mRNA and protein in NLRP₃ inflammasomes including NLRP₃ mRNA, ASC mRNA, caspase-1 mRNA and ASC protein levels increased in the hyperuricaemia and gouty nephropathy groups compared with the control group. Importantly, the elevated levels of IL-1 β and IL-18 indicated that NLRP₃ inflammasomes were activated in hyperuricaemia and gouty nephropathy. The expression of ASC and caspase-1 mRNA and protein was higher in the gouty nephropathy group than in the hyperuricaemia group with the level of IL-1 β also being significantly increased. The results indicated that the NLRP₃ inflammasome was activated and induced the polymerization of the adaptor molecule ASC, leading to the production of IL-1 β . Taken together, these results indirectly indicated that the NLRP₃ inflammasome has a pivotal role in the progression of gouty nephropathy.

Although the specific activation mechanism of the NLRP₃ inflammasome is unclear, recent studies have proposed three hypotheses regarding the activation of NLRP₃ inflammasomes:

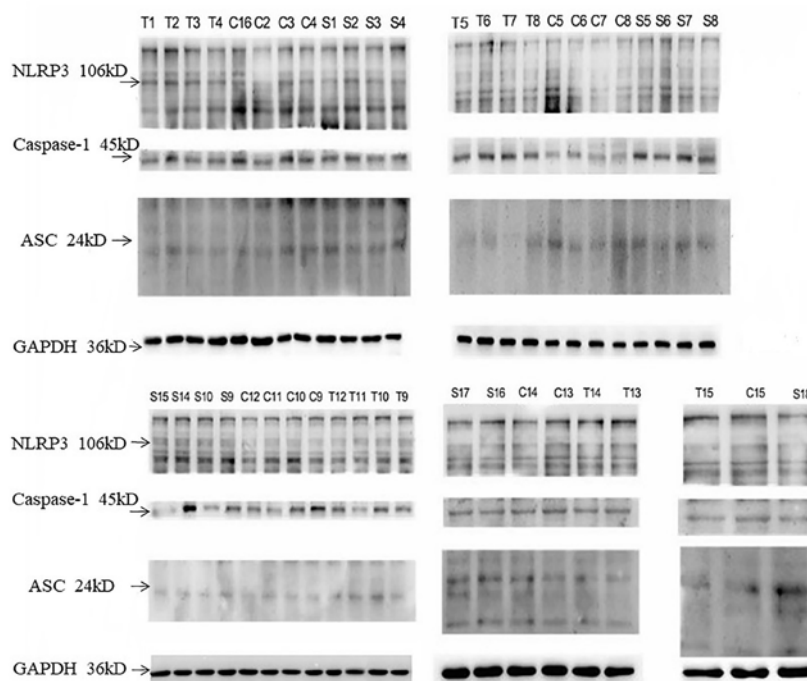


Figure 4. Expression of NLRP₃, ASC and caspase-1 protein in peripheral blood mononuclear cells. Representative western blots of peripheral blood mononuclear cells isolated from control, hyperuricemia and gouty nephropathy patients. NLRP₃, Nod-like receptor protein 3; ASC, apoptosis-associated speck like protein; C, control group; T, hyperuricemia group; S, gouty nephropathy group.

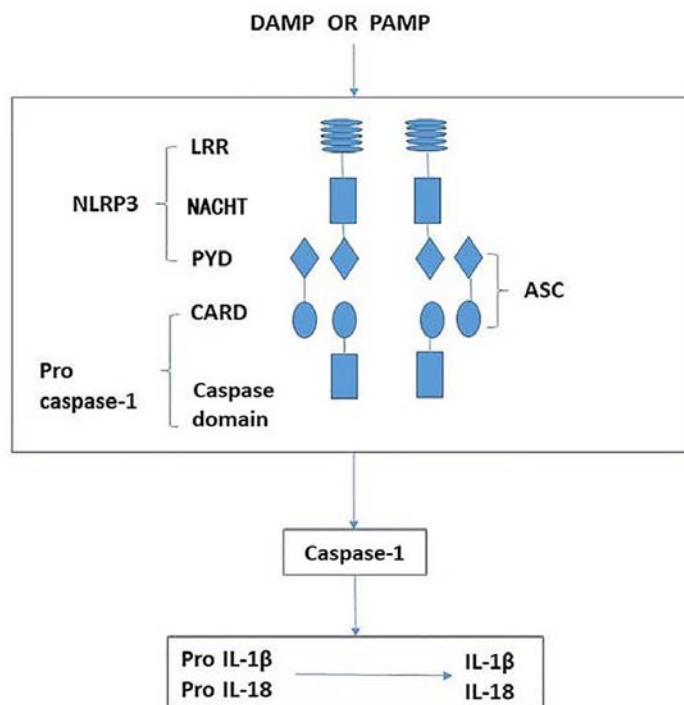


Figure 5. Schematic demonstrating the activation of NLRP₃ leading to release of proinflammatory factors. The NLRP₃ inflammasome is comprised of NLRP₃, ASC and caspase-1. When sensing danger signals, including PAMP and DAMP, NLRP₃ oligomerizes via homotypic interactions between NACHT domains. The PYD of NLRP₃ is then exposed for interaction with the PYD of ASC. The CARD of ASC in turn recruits pro-caspase-1 through CARD-CARD interactions, resulting in activated caspase-1. Activated caspase-1 then cleaves intracellular IL-1 β and IL-18 precursors to form mature IL-1 β and IL-18, which are secreted into the extracellular matrix. NLRP₃, nucleotide-binding domain and leucine-rich repeat protein 3; ASC, apoptosis-associated speck like protein; LRR, leucine-rich repeat; NACHT, nucleotide-binding and oligomerization domain; PYD, pyrin domain; CARD, caspase recruitment domain; IL-1 β , interleukin-1 β ; IL-18, interleukin-18; PAMP, pattern-associated molecular pattern; DAMP, danger-associated molecular pattern.

Firstly, all bacteria, viruses, particles or crystals can stimulate cells to produce reactive oxygen species, which can activate

the NLRP₃ inflammasome (29). In the course of oxidative stress, thioredoxin, following its own oxidation, releases

Table IV. Expression of NLRP₃, ASC and caspase-1 mRNA in peripheral blood mononuclear cells.

Group	n	NLRP ₃	ASC	Caspase-1
Control	15	23.54±1.19	25.88±1.18	26.99±1.43
Hyperuricemia	15	26.05±1.89 ^a	27.49±3.56 ^a	28.05±2.41 ^a
Gouty nephropathy	15	25.43±1.66 ^a	32.21±3.01 ^{a,c}	30.80±2.80 ^{a,b}

Values are presented as the mean ± standard deviation. ^aP<0.01 vs. the control group; ^bP<0.05 and ^cP<0.01 vs. the hyperuricemia group; NLRP₃, nucleotide-binding domain and leucine-rich repeat protein 3; ASC, apoptosis-associated speck like protein.

Table V. Expression of NLRP₃, ASC, caspase-1 protein in peripheral blood mononuclear cells.

Group	n	NLRP ₃ /GAPDH	ASC/GAPDH	Caspase-1/GAPDH
Control	15	0.45±0.18	0.22±0.08	0.57±0.16
Hyperuricemia	15	0.55±0.12	0.26±0.11 ^a	0.57±0.16
Gouty nephropathy	15	0.60±0.16 ^b	0.47±0.19 ^{a,c}	0.78±0.15 ^{b,c}

Values are presented as the mean ± standard deviation, GAPDH was used as an internal reference. ^aP<0.05 and ^bP<0.01 vs. the control group; ^cP<0.05 vs. the hyperuricemia group. NLRP₃, nucleotide-binding domain and leucine-rich repeat protein 3; ASC, apoptosis-associated speck like protein.

Table VI. Expression of IL-1β and IL-18 in plasma of patients.

Group	n	IL-1β (pg/ml)	IL-18 (pg/ml)
Control	15	28.41±14.05	323.35±96.16
Hyperuricemia	15	55.19±16.79 ^a	718.16±211.42 ^a
Gouty nephropathy	15	81.20±17.63 ^{a,b}	870.67±371.13 ^a

Values are mean ± standard deviation. ^aP<0.01 vs. control group; ^bP<0.01 vs. hyperuricemia group. IL-1β, interleukin-1β; IL-18, interleukin-18.

thioredoxin-interacting protein, activating the NLRP₃ inflammasome (30). Secondly, crystals or particles, such as MSU, silicon dioxide, amyloid and cholesterol crystals, are phagocytosed by macrophages into lysosomes. This accumulation causes lysosomal rupture and the release of cathepsin B and other proteases, inducing the activation of the NLRP₃ inflammasome (31,32) Finally, stimulation induces cells to release ATP on the cell surface of the P2X7 purine receptor, triggering the potassium ion outflow mechanism and calcium wave transport mechanism leading to mitochondrial damage. This releases mitochondrial DNA (mtDNA) and activates the NLRP₃ inflammasome (33,34). Previous studies have demonstrated that NLRP₃ can identify uric acid crystals and other dangerous signals (3,35). Uric acid crystals trigger IL-1β-mediated inflammation by activating the NLRP₃ inflammasome via the aforementioned three mechanisms (36).

The present study hypothesized that MSU acts as an endogenous DAMP, contributing to the progression of the NLRP₃ inflammasome in hyperuricaemia and gouty nephropathy. When high levels of uric acid in the body cause uric acid crystals to be deposited in tissues, macrophages directly

phagocytose urate crystals, which are too large to be efficiently cleared and are likely to induce the production of ROS on their way to becoming lysosomes (9). Following the phagocytosis of MSU, macrophages form phagocytic bodies and the subsequent intracellular rupture of these bodies releases cathepsin B, which activates the NLRP₃ inflammasome (37). MSU also activates the NLRP₃ inflammasome by causing intracellular potassium efflux (38), leading to mitochondrial damage. Furthermore, by releasing mtDNA, the maturation and release of IL-1β and IL-18 is stimulated. A previous study determined that uric acid activates NLRP₃ inflammasomes via mitochondrial ROS production and their results demonstrated the direct role of hyperuricaemia in activating NLRP₃ inflammasomes in macrophages (39). Berberine may influence the NLRP₃ inflammasome and be involved in MSU crystal-induced innate immune responses, attenuating the expression of NLRP₃, further inhibiting the downstream signalling of molecular IL-1β and eventually having a role in the treatment of gouty arthritis (40). The present study observed that gouty nephropathy is associated with elevated levels of uric acid in serum and high expression of NLRP₃, ASC and caspase-1 mRNA and protein, as well as IL-1β and IL-18 expression. The present results indicated that uric acid-induced NLRP₃ inflammasomes are associated with gouty nephropathy with the underlying mechanism potentially involving one of the aforementioned three hypotheses.

The present study has limitations as the exact association between NLRP₃ inflammasomes and gouty nephropathy was not elucidated. Further studies are therefore required to explore the role of the NLRP₃ inflammasome in humans and *in vivo*. In addition to the need for large clinical trials, more studies are required to better understand the biology of uric acid. To the best of our knowledge, clinical study into the regulation mechanism of the NLRP₃ inflammasome signalling pathway with regards to gouty nephropathy has not been performed.

In conclusion, the present study demonstrated that the NLRP₃ inflammasome was associated with the progression of hyperuricaemia and gouty nephropathy, leading to the inflammatory response and renal damage. Although the precise mechanism of the NLRP₃ inflammasome signalling pathway and the means by which MSU triggers the inflammasome remains a matter of debate, the importance of the NLRP₃ inflammasome in gouty nephropathy has been established. The present study proposes that future therapeutic strategies for gouty nephropathy should be based on blocking uric acid or inhibiting its activation of the NLRP₃ inflammasome. Further studies will be required to explore the mechanism of how uric acid activates the NLRP₃ inflammasome to allow for the early diagnosis of gouty nephropathy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YZZ and XLS designed the experiments, analyzed the data and wrote the manuscript. YPX and YZZ performed the experiments. FJG and ASZ analyzed and interpreted the data. JHC contributed to the design of the experimental methods, wrote the manuscript and approved the version to be published and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Affiliated Bao'an Hospital of Shenzhen (approval no. BYL2016001) and written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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