

Association of sirtuin 1 gene polymorphisms with nephrolithiasis in Eastern Chinese population

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ABSTRACT

Sirtuin 1 (SIRT1), an NAD⁺-dependent deacylase, has been identified to be associated with renal tubular inflammatory conditions and metabolic disorders, which are risk factors of nephrolithiasis. To further confirm the role of the SIRT1 in kidney stone formation, the expression of SIRT1 was analyzed based on a mouse model and the genetic polymorphisms of *SIRT1* gene was compared between patients with kidney stones and controls. The calcium oxalate (CaOx) crystal-induced renal injury model was established to analyze the expression of SIRT1 in the kidney tissue of both wild-type and ApoE(–/–) mice. And a total of 430 Eastern Chinese subjects (215 patients with nephrolithiasis and 215 age- and gender-matched controls) were recruited for the present study to investigate the associations between 6 common single nucleotide polymorphisms (SNPs) (i.e., rs10509291, rs3740051, rs932658, rs33957861, rs3818292 and rs1467568) in the *SIRT1* gene and the incidence of kidney stones. Pairwise linkage disequilibrium and the haplotypes of the 6 SNPs were also analyzed. The genotypes of SIRT1 gene polymorphisms were analyzed by a Snapshot assay. Reduced expression of SIRT1 was observed in the kidney of the mice in the crystal group, revealing the potential role of SIRT1 in the nephrolithiasis. However, we did not find a significant association between the 6 SNPs of the SIRT1 gene and kidney stone formation in the Eastern Chinese population.

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1. Introduction

The worldwide prevalence of kidney stone disease, also known as nephrolithiasis, has increased in recent years from 5% to 12% [1,2], endangering the health of the public and the quality of life of patients. The formation of kidney stones may lead to a decline in renal function [3] and even persistent kidney damage, thereby increasing the risk of acute or chronic kidney disease, especially after recurrent attacks [4]. Furthermore, kidney stones are indicated to be associated with cardiovascular diseases including coronary heart disease and stroke [5,6]. Therefore, nephrolithiasis is increasingly recognized as a systemic disorder.

Approximately 70% of stones are composed of calcium oxalate (CaOx); other contents include calcium phosphate, uric acid and other salts [7]. Most calcium oxalate (CaOx) stones develop from sub-epithelial plaques of calcium phosphate (CaP), known as

Randall's plaques, which are also scattered in the interstitium and around the collecting ducts and blood vessels [8]. In addition to abnormal mineral metabolism, oxidative stress (OS), inflammation and aberrant crystallization inhibition play significant roles in the deposition of CaP in the basement membrane of renal tubules or vessels [9]. This plaque deposition further aggravates OS, causing injury to tubular epithelial cells. Renal injury and the normal aging process are proposed to promote this process. With the aggregation and calcification of plaques, oxidative and inflammatory reactions subsequently promote the further formation of stones [10]. Cell injury, inflammation, OS, interstitial fibrosis and intratubular crystal deposition have been observed in renal biopsies during stone formation at all stages and promote the overall process [11,12]. According to research, the pathophysiology of urinary stone

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formation is complex and involves metabolic, genetic, and environmental factors [13].

As a protein member of a conserved family of NAD(+)-dependent deacylases called sirtuins, sirtuin1 (SIRT1) is associated with numerous cellular signaling pathways that are mainly involved in cytoprotective effects and metabolic regulation [14]. Increasing evidence shows that reduced SIRT1 levels are closely associated with various inflammatory diseases. Therefore, pharmacologic activation of SIRT1 could be a promising therapeutic strategy for OS- or inflammation-related diseases [15]. In the kidneys, SIRT1 may inhibit OS, inflammation and renal cell apoptosis. The renal protective effects of SIRT1 have been found in various models of renal disorders, including diabetic nephropathy, acute kidney injury, chronic kidney disease and lupus nephritis. In cisplatin-induced renal injury, SIRT1 inhibits oxidative stress by upregulating catalase expression and increasing the number and functions of mitochondria [16,17]. In addition to protective effects in tubular cells, SIRT1 also exhibits antioxidative and anti-apoptosis activity in vascular endothelial cells [18]. Considering that vessels and tubules under conditions of OS represent the origins of plaques, we proposed a potential association between SIRT1 and the pathogenesis of nephrolithiasis. In addition, kidney fibrogenesis was observed with the calcification and formation of calcium crystals in the kidney (Hu et al., 2015), and SIRT1 up-regulation induced by an SIRT1 activator can attenuate renal fibrosis and renal injury [19,20]. Therefore, SIRT1 is considered a new potential therapeutic target for kidney stones and warrants further investigation.

Understanding the genetic basis of complicated human diseases such as nephrolithiasis has been increasingly emphasized in medical research. In this study, the role of *SIRT1* gene in nephrolithiasis was firstly explored based on a mouse model of CaOx crystal-induced renal injury. In addition, genetic variations of the *SIRT1* gene have been found to be associated with cardiovascular diseases [21] and chronic inflammatory states [22]. To confirm whether there is a correlation between *SIRT1* and the risk of kidney stones, the associations between single nucleotide polymorphisms (SNPs) of the *SIRT1* gene and the incidence of nephrolithiasis in the patients were investigated in the present study.

2. Material and methods

2.1. Experimental animals

Twelve wild-type male C57BL/6 mice (7–8 weeks old) were purchased from the Shanghai SLAC Animal Co.,

Ltd. (Shanghai, China). Twelve male ApoE KO (ApoE^{-/-}) mice of the same age were acquired from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. After conditioned housing for one week, wild-type and ApoE KO mice were respectively and equally divided into the control and the crystal model groups with 6 mice each. The crystal group was administered glyoxylate (100 mg/kg/day) by intraperitoneal injections for 3 consecutive days, and the control group was intra-abdominally injected daily with normal-volume saline (20 mL/kg/day) per day as previously described [23]. On day 3 after the administration of glyoxalate, kidney samples were collected, and the cortex and medulla junction tissue were dissected to be further analyzed. All animal studies were performed in accordance with the National Institutes of Health (NIH) guide for the Care and Use of Laboratory Animals. The experimental procedures were approved by the Ethical Committee for the Experimental Use of Animals at Second Military Medical University (Shanghai, China).

2.2. Western blot

Western blotting was conducted following previous method [23]. Harvested corticomedullary tissues from different groups were respectively homogenized in lysis buffer containing protease inhibitor and phosphatase inhibitor. The homogenates were centrifuged at 12,000 rpm for 5 min at 4 °C and the supernatant was collected. The protein concentration in each lysate was determined using a BCA protein assay kit (Thermo Fisher Scientific). Equal amount of each sample was subjected to SDS-PAGE gel for separation and transferred onto a nitrocellulose membrane (GE Healthcare Life Sciences). After blocking, the membrane was incubated with rabbit polyclonal anti-SIRT1 antibodies (1:1000, Abcam) at 4 °C overnight. After washing with TBST, the membrane was incubated with a fluorescence-conjugated secondary anti-rabbit antibody (1:10000, Licor) for 60 min at room temperature. The signals were visualized using the Odyssey Infrared Imaging System (Licor, NE, USA) and quantitatively analyzed by normalizing to β -actin using the Image J software (National Institutes of Health, Bethesda, MD, USA).

2.3. Clinical subjects

Here, we report a hospital-based case-control study with 215 patients who underwent urolithiasis surgery for kidney stones from eastern China at Changhai Hospital in Shanghai. The diagnosis of stones was confirmed by plain X-ray film and renal ultrasound. After

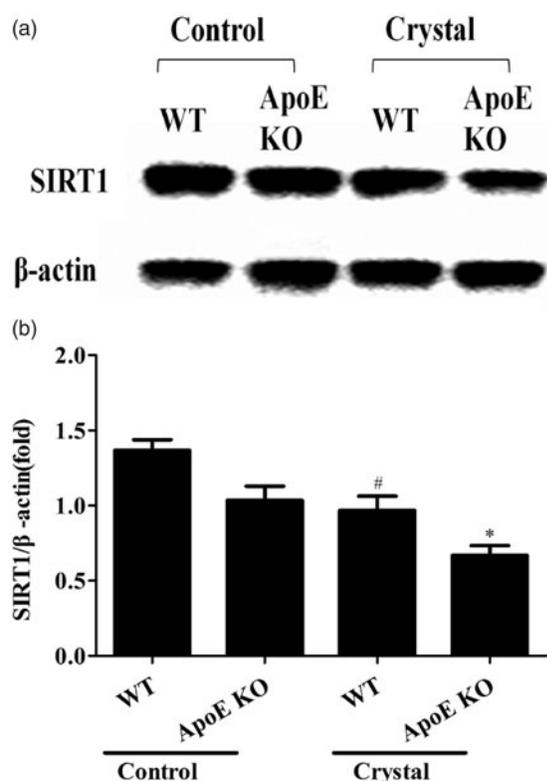


Figure 1. The expression of SIRT1 in the kidney tissue of mice. Western blot for SIRT1 protein in the corticomedullary tissue of kidney were analyzed based on both wild-type(WT) mice and ApoE KO mice. β -actin was used as loading control. Data were expressed as mean \pm SEM (n = 6). *compared with the WT mice in the control group, $P < 0.05$; #compared with the ApoE KO mice in the control group, $P < 0.05$.

In all tests, two-tailed values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Reduced expression of SIRT1 in the mice of crystal group

As could be seen from Figure 1, the expression of SIRT1 were significantly decreased in the kidney tissue of crystal groups compared to the controls based on both wild-type and ApoE KO mice. And the difference between the expression of SIRT1 in wild-type and ApoE KO was not significant.

3.2. Demographic characteristics of the patients and controls

In total, 430 subjects participated in this study, including 215 patients with calcium-containing kidney stones and 215 age- and sex-matched controls. The baseline characteristics and clinical data of the patients and controls are presented in Table 2. Males accounted for 123

Table 2. Demographic and characteristics of the nephrolithiasis patients and controls.

Characteristics	Controls (n = 215)	Nephrolithiasis patients (n = 215)	P
Gender (Male/Female)	123/92	123/92	1.000
Age (years)	46.85 \pm 12.56	45.86 \pm 8.76	0.343
BMI (Kg/m ²)	23.39 \pm 1.96	23.87 \pm 3.13	0.057
Obesity No.	7	8	1.000
Hypertension No.	96	109	0.247
DM No.	61	65	0.751
Hyperlipidemia No.	27	29	0.886

(57.2%) subjects in both groups. The average age of the patients with nephrolithiasis was 46.85 years, which was not significantly different from that of the controls. BMI was not significantly different between the groups, with a value of 23.87 \pm 3.13 kg/m² for the nephrolithiasis group compared to 23.39 \pm 1.96 kg/m² for the control group. No significant differences were noted in the distribution of hypertension, diabetes mellitus (DM) or hyperlipidemia in the two groups.

3.3. Distributions of alleles and genotype frequencies

Six SNPs (i.e., rs10509291, rs3740051, rs932658, rs33957861, rs3818292 and rs1467568) of the *SIRT1* gene were selected as tags for analysis. The genotypic distribution of each SNP was consistent with HWE for the control group ($p = 0.368, 0.368, 0.052, 0.227, 0.368$ and 0.052 , respectively). The frequency distributions of each SNP genotype and allele in the patients and controls are summarized in Table 3. No significant association was observed between nephrolithiasis and genotypic distribution in the two groups by the Chi-square test. Furthermore, the logistic regression analysis with adjustments for age and gender also revealed no significant risk association between nephrolithiasis and each SNP.

Pairwise linkage disequilibrium indicated that these 6 SNPs exhibited obvious linkage disequilibrium in one haplotype block (rs10509291, rs3740051, rs932658, rs33957861, rs3818292 and rs1467568) in this region (Figure 2). In the haplotype analysis (Table 4), by comparing the distribution frequencies between the patients and control subjects, we found no significant differences in effects for any haplotypes in *SIRT1*.

4. Discussion

The human *SIRT1* gene is located in the chromosome region 10q21.3 [24] and its alternative splicing results in multiple transcript variants. As a human sirtuin, SIRT1 may function as an intracellular regulatory protein via

Table 3. Genotypic distribution of the SIRT1 gene variants.

SNP sites	case	controls	<i>P</i> *	OR†	<i>P</i> †
rs10509291					
Genotype					
TT	114 (53.0%)	123 (57.2%)	0.215	1	
TA	91 (42.3%)	76 (35.3%)		1.32 (0.88-1.97)	0.175
AA	10 (4.7%)	16 (7.4%)		0.67 (0.29-1.55)	0.343
Allele					
T	319 (74.2%)	322 (74.9%)	0.812	1	
A	111 (25.8%)	108 (25.1%)		1.05 (0.77-1.43)	0.780
rs3740051					
Genotype					
AA	117 (54.4%)	123 (57.2%)	0.299	1	
GA	88 (40.9%)	76 (35.3%)		1.24 (0.83-1.85)	0.288
GG	10 (4.7%)	16 (7.4%)		0.65 (0.28-1.50)	0.321
Allele					
A	322 (74.9%)	322 (74.9%)	1.000	1	
G	108 (25.1%)	108 (25.1%)		1.01 (0.74-1.38)	0.965
rs932658					
Genotype					
AA	154 (71.6%)	145 (67.4%)	0.414	1	
CA	57 (26.5%)	68 (31.6%)		0.79 (0.52-1.20)	0.259
CC	4 (1.9%)	2 (0.9%)		1.88 (0.34-10.449)	0.473
Allele					
A	365 (84.9%)	358 (83.3%)	0.496	1	
C	65 (15.1%)	72 (16.7%)		0.87 (0.59-1.28)	0.483
rs33957861					
Genotype					
CC	171 (79.6%)	175 (81.4%)	0.200	1	
CT	40 (18.6%)	40 (18.6%)		1.03 (0.63-1.67)	0.920
TT	4 (1.9%)	0 (0.0%)		/	0.999
Allele					
C	382 (88.8%)	390 (90.7%)	0.366	1	
T	48 (11.2%)	40 (9.3%)		1.23 (0.79-1.92)	0.362
rs3818292					
Genotype					
AA	114 (53.0%)	123 (57.2%)	0.211	1	
GA	91 (42.3%)	76 (35.3%)		1.32 (0.88-1.97)	0.175
GG	10 (4.7%)	16 (7.4%)		0.67 (0.29-1.54)	0.343
Allele					
A	319 (74.2%)	322 (74.9%)	0.812	1	
G	111 (25.8%)	108 (25.1%)		1.05 (0.77-1.43)	0.780
rs1467568					
Genotype					
AA	154 (71.6%)	145 (67.4%)	0.585	1	
GA	58 (27.0%)	68 (31.6%)		0.80 (0.53-1.22)	0.295
GG	3 (1.4%)	2 (0.9%)		1.40 (0.23-8.54)	0.720
Allele					
A	366 (85.1%)	358 (83.3%)	0.431	1	
G	64 (14.9%)	72 (16.7%)		0.85 (0.58-1.26)	0.419

*Chi-square test.

†Logistic regression analysis after adjustment with age and sex.

NAD⁺-dependent deacetylase activity. SIRT1 has been observed in both the nucleus and the cytoplasm where it interacts with nuclear and cytosolic proteins in different roles [25]. SIRT1 is expressed in a wide range of tissues and organs in humans, with relatively high expression levels in the fat, kidneys and liver [26]. SIRT1 protects cells by regulating metabolism and exerting anti-apoptotic, anti-oxidative and anti-inflammatory effects against injury in renal cells and in cells from other tissues. The role of SIRT1 in the renal interstitial fibrosis have been concentrated these years. Tubulointerstitial fibrosis and decreased Sirt1 expression was found in the kidney of unilateral ureteral

obstruction rats [27]. And the activation of SIRT1 was demonstrated to attenuate the inflammation and inhibit the oxidative stress in renal injury [28,29]. In present study, the association between nephrolithiasis and the expression of SIRT1 was firstly explored based on the mouse model of CaOx crystal-induced renal injury. According to previous researches, the level of SIRT1 could be regulated by the type or the knockout of ApoE [30,31]. But the different expression of SIRT1 in kidney was not found between the wild-type mice and the ApoE KO mice in this study. The reduced expression of SIRT1 in the crystal group was both observed in the wild-type mice and the ApoE KO mice, revealing the potential role of SIRT1 in the nephrolithiasis.

Given the accumulating evidence indicating the close association between SIRT1 and various diseases, genetic variations of *SIRT1* have been widely investigated. Several genetic polymorphisms of *SIRT1* have also been shown to be associated with different health conditions, such as metabolic disorders (obesity, hyperglycemia and hypertension) [21,32,33] and chronic inflammatory states [22]. Systemic inflammation [34], urine supersaturation [35] and renal injury induced by metabolic disorders may promote the formation of stones. However, the correlation between *SIRT1* polymorphisms and nephrolithiasis has not been studied.

Several genetic polymorphisms have been found to play important roles in the pathogenesis of calcium nephrolithiasis, including SNPs in transient receptor potential vanilloid member 5 (TRPV5) [36], calcitonin receptor gene (CALCR) [37] and vitamin D receptor (VDR) [38], which are related to the maintenance of calcium homeostasis or the matrix protein. SIRT1 enzymatically potentiates 1,25-dihydroxyvitamin D3 signaling via VDR deacetylation and is also considered a positive regulator of osteoblasts [39] and osteogenic differentiation [40]. Given the important role of *SIRT1* gene in calcium metabolism, genetic polymorphisms of *SIRT1* are considered potential impact factors for hypercalciuria, which leads to nephrolithiasis [41].

In this study, 6 common non-coding SNPs of *SIRT1* were selected as tags for analysis to explore the relationship between the *SIRT1* gene and kidney stone formation. Among them, the rs10509291, rs3740051, and rs932658 polymorphisms of the *SIRT1* gene were 5'-flanking and primarily function in the regulation of gene transcription. The rs33957861 (intron1), rs3818292 (intron5) and rs1467568 (intron8) polymorphisms are *SIRT1* gene intron variants. These 6 polymorphisms of SIRT1 have been widely investigated mainly in the context of metabolism and have been found to be associated with the risk of obesity (rs10509291, rs33957861,

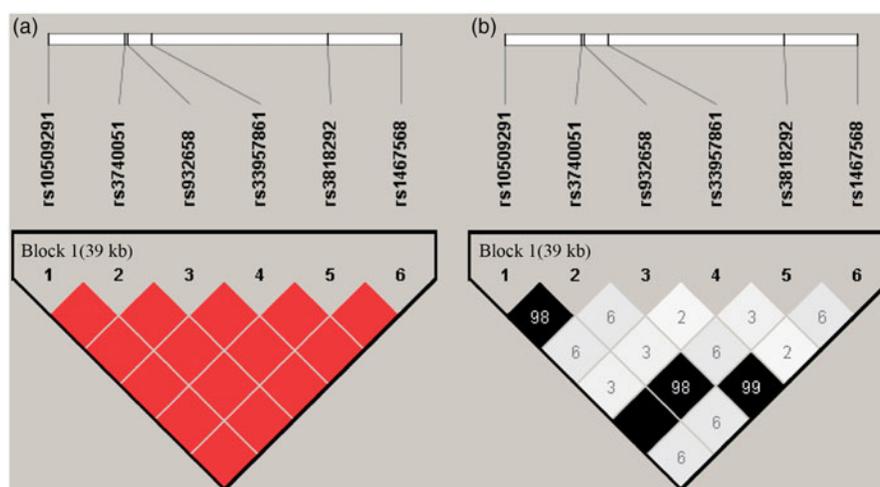


Figure 2. Linkage disequilibrium maps for SNPs genotyped in *SIRT1* region. (A) Shades of red demonstrate the strength of the pairwise linkage disequilibrium based on D' , and numbers represent the value of D' expressed as a percentage. The blanks represent $D'=1$. (B) Shades of gray show the strength of the pairwise linkage disequilibrium based on r^2 , and numbers indicate the value of r^2 expressed as a percentage.

Table 4. Associations of one haplotype in *SIRT1* region with kidney stone.

Haplotype	Haplotype frequencies		P value
	Nephrolithiasis	Control	
Block*			
TACCAG	0.150	0.167	0.491
AGACGA	0.254	0.251	0.937
TAATAA	0.112	0.099	0.370
TAACAA	0.485	0.488	0.914

*rs10509291; rs3740051; rs932658; rs33957861; rs3818292; rs1467568.

and rs1467568) [32,42,43] and carbohydrate metabolism disturbances (rs10509291 and rs1467568) [44,45]. In this study, the distribution of obesity, hypertension, diabetes and hyperlipidemia was matched between control and patient samples, with no significant differences. Therefore, we could focus on the effects of *SIRT1* gene in nephrolithiasis locally.

According to previous research, rs3740051 has been widely studied and its polymorphism was found to be associated with the expression of *SIRT1*. Female cancer patients carrying the G minor allele of rs3740051 exhibited higher serum *SIRT1* levels [46]. Given the beneficial role of *SIRT1* in oxidative reactions and cell injuries [47], the G allele for rs3740051, which elicits high *SIRT1* levels, was hypothesized as a protective factor. However, the frequency of the genotype with the G allele in the stone group was nearly the same as that of the control group in our study. More studies are needed to explore whether SNPs are correlated with the expression of *SIRT1* in kidney tissues. Rs3740051 and rs1467568 have been indicated to be associated with carotid atherosclerosis, especially in women [48]. The variant of

rs932658 reportedly alters the transcriptional activities of the *SIRT1* gene promoter and changes *SIRT1* levels, contributing to hernia development. This phenomenon was attributed to the regulatory roles of *SIRT1* gene in the differentiation of human cells [49]. In general, SNPs of *SIRT1* play different and complicated roles in different diseases. A direct effect of *SIRT1* gene in kidney stone formation was not found in the present study.

In conclusion, the reduced expression of *SIRT1* was observed in the injured kidney of the mice induced by CaOx crystal, revealing the potential role of *SIRT1* in the nephrolithiasis. In addition, we genetically analyzed 6 SNPs of the *SIRT1* gene in kidney stone patients and controls. However, no significant association was found between genetic polymorphisms and the development of nephrolithiasis. Participants in our study were of Eastern Chinese descent. Due to racial differences, various altered genotype distributions may be present in other populations. Further studies with larger sample sizes are required to explore the potential relationship between *SIRT1* and nephrolithiasis.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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