Int J Clin Exp Pathol 2016;9(1):256-266
www.ijcep.com /ISSN:1936-2625/IJCEP0017221

Original Article

Genetic variants in renal interstitial inflammation-related genes CSF-1 and CD44 are associated with susceptibility of nephrolithiasis

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Received October 2, 2015; Aaccepted November 22, 2015; Epub January 1, 2016; Published January 15, 2016

Abstract: Previous studies showed that interstitial inflammation was more prevalent among CaOx stone formers (SF), indicating interstitial inflammation might be an important component part of pathologic processes in CaOx formation. However, contribution of genetic variants in interstitial inflammation-related CD44 and CSF-1 towards the risk of nephrolithiasis has not been reported. In this study, 582 patients and a group of 499 stone-free controls were enrolled in a case-control study. Four SNPs in CD44 and CSF-1 were selected to probe associations with nephrolithiasis. Significant differences were observed in the distribution of the genotype and allele frequencies of rs13347 and rs2050462 between the nephrolithiasis patients and control subjects. Our study suggested that the CSF-1 rs2050462 C allele and CD44 rs13347 T allele were associated with susceptibility of nephrolithiasis. Through stratification analyses, we probed that the associations between CSF-1 rs2050462 and CD44 rs13347 with nephrolithiasis risk were more prominent in some specific subgroups. Individuals carrying ≥2 risk alleles were more susceptible to nephrolithiasis. In conclusion, our study proved that the genetic variants in interstitial inflammation-related genes CD44 and CSF-1 played an important role in the susceptibility of nephrolithiasis.

Keywords: Genetic variants, CSF-1, CD44, renal interstitial inflammation, macrophages, nephrolithiasis susceptibility

Introduction

Nephrolithiasis is one of the most common disorders around the world, which is a multifactorial disease with genetic and environmental factors determining the likelihood of stone formation [1]. Through stone composition analysis, about 75% to 80% of renal stones are calcium oxalate (CaOx) stone, including monohydrate (COM) and dehydrate (COD) forms [2]. The pathogenesis of calcium oxalate remains unclear although some putative pathogenic hypotheses and observations have been made over the last 3 decades. Several researches have aimed at the possible pathogenic mechanism of CaOx stone formation associated with renal crystal deposition on the cellular composition of the renal interstitium [3-5]. Study by Ruud et al. showed that interstitial CaOx crystals can be removed and that monocytes, macrophages (Mφs), and multinucleate giant cells are likely involved in this process in Rats. Macrophages and multinucleated giant cells are the major cells that encapsulate the interstitial crystals in both rat and humans [6]. Based on the above, we hypothesized macrophages and multinucleated giant cells may play a special role in crystal retention.

Colony-stimulating factor 1 (CSF1, also referred to as macrophage CSF or M-CSF) has been found to be an essential factor for the growth, survival, and differentiation of monocyte/macrophages [7]. CSF-1 is known to be required for the production and maintenance of many tissue macrophage populations [8]. CSF-1 signaling mediates tissue regeneration after injury and also, alters Mφ polarization to the M2 phenotype [9-11]. CSF-1 deficiency may result in increased renal crystal deposition and fewer M2-like Mφs [12].

CD44 molecule, a receptor for hyaluronic acid (HA), encode by CD44 gene, is a transmembrane protein [13, 14]. The protein can interact with osteopontin (OPN), a major component in the urinary stone matrix that inhibits nucleation, growth, and aggregation of CaOx crystals [15, 16]. CD44 were also related to tissue inflammation and inflammatory-related Mφ migration. Inhibiting CD44 could reduce renal crystal formation by increasing M2 phagocytic activity [12].

These observations showed that CD44 and CSF-1 were all related to tissue inflammation and inflammatory-related Mφ polarization and migration, affecting renal crystal formation by increasing or reducing M2 phagocytic activity. However, the contribution of genetic variants in interstitial inflammation-related CD44 and CSF-1 towards the risk of nephrolithiasis has not been reported. To verify this hypothesis, we selected four potentially functional single nucleotide polymorphisms (SNPs) in CSF-1 and CD44 to probe if these genetic variants associated with CaOx nephrolithiasis susceptibility.

Patients and methods

Study population

This study was approved by the Institutional Review Board of the Nanjing Medical University, Nanjing, China. In total, 582 patients and a group of 499 stone-free controls were enrolled in the case-control study from September 2013 to May 2015 at The First Affiliated Hospital of Nanjing Medical University, Nanjing, China. All subjects are ethnic Han Chinese from different families and have no blood relationship in this study. All these stone samples (768 patients) were obtained either from surgery (such as percutaneous nephrolithotomy (PCNL), laparoscopy and ureteroscopy) or extracorporeal shock wave lithotripsy (ESWL), which were analyzed through the Infrared Spectroscopy method postoperatively. Through analysis of stone composition, 582 patients with pure calcium oxalate stones (COM or COD) or combined forms were selected in this study. The controls were recruited from volunteer healthy subjects who were seeking health care in the outpatient departments at the hospital and were frequency matched to the cases on sex and age (±5 years). All these controls were unrelated to the cases and had no individual history or radiological finding of urolithiasis. A standard questionnaire was administered through face-to-face interviews by trained interviewers to collect demographic data and related factors before recruitment. All subjects donated 5 ml venous blood after signing an informed consent. The response rate for both case and control subjects was >85%.

SNPs selection

All the SNPs were selected by using the genotype data obtained from unrelated Han Chinese in Beijing individuals in the Hap Map database (http://hapmap.ncbi .nlm.nih.gov/). Each of the potentially functional polymorphisms should locate in the 5' flanking regions, 5' un-translated region (UTR), 3' UTR, or coding regions and have a minor allele frequency (MAF) >5% in Han Chinese in Beijing. During the SNPs Selection, linkage disequilibrium was considered. If some of the SNPs were in complete linkage disequilibrium (r^2=1), only one SNP will be chosen for genotyping. Finally, we included two SNPs (rs-333951 in 5' flanking region and rs2050462 in 3' UTR) in CSF-1 and two SNPs (rs13347 and rs8193 in 3' UTR) in CD44.

DNA extraction and genotyping

Genomic DNA was isolated from the peripheral blood by proteinase K digestion and phenol/chloroform extraction. The genotyping of the four polymorphisms was carried out using predesigned TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). Amplifications and analysis were carried out in the 384-well ABI 7900HT Real-Time PCR System (Applied Biosystems), using the SDS software 2.3 for allelic discrimination (Applied Biosystems). Controls were included to ensure accuracy of genotyping in each plate. About 10% of the samples were randomly selected for repeated genotyping, and the results were 100% concordant.

Statistical analyses

Before analysis, Hardy-Weinberg equilibrium was tested using a goodness-of-fit chi-square test. The chi-square test (for categorical variables) and the student's t-test (for continuous variables) were used to compare the differences in the distributions of demographic characteristics, selected variables, and frequencies of genotypes between cases and controls. The associations between risk of urolithiasis and

Table 1. Frequency distributions of selected variables between the nephrolithiasis cases and controls

Characteristics	Case (n=582)		Control (n=499)		P[a]
	n	%	n	%	
Age (years) Mean ± SD	50.8±9.6		50.3±11.7		0.772
BMI (kg/m^2) Mean ± SD	24.3±3.1		24.2±3.1		0.865
Sex					
Male	382	65.64	331	66.33	0.810
Female	200	34.36	168	33.67	
Smoking status					
No (ever)	399	68.56	338	67.74	0.773
Yes (never)	183	31.44	161	32.26	
Pack-year of smoking					
0	399	68.56	338	67.74	0.127
<20	136	23.37	134	26.85	
≥20	47	8.08	27	5.41	
Drinking status					
No	411	70.62	329	65.93	0.098
Yes	171	29.38	170	34.07	
Family history of urolithiasis					
No	537	92.27	477	95.59	**0.024**[b]
Yes	45	7.73	22	4.41	
Hypertension					
No	463	79.55	402	80.56	0.680
Yes	119	20.45	97	19.44	
Diabetes					
No	551	94.67	485	97.19	
Yes	31	5.33	14	2.81	**0.039**[b]

[a]Two-sided χ^2 test for the frequency distributions of selected variable between cases and controls. [b]Bold values indicated significant differences between two groups.

polymorphisms were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from unconditional logistic regression analysis with the adjustment for possible confounders. All data were analyzed with the software SATA 12.0 with two-sided test, and the adjusted *P* value of less than 0.05 was considered to be statistically significant.

Results

Characteristics of the study population

The frequency distributions of selected characteristics of 1081 subjects were presented in **Table 1**. No significant differences were observed between the cases and controls with regarding to age, body mass index, sex, smoking status, pack-year of smoking, drinking status, and hypertension (all P>0.05). However, there were more Diabetes mellitus patients in the cases than in the controls (5.33% versus 2.81%, P=0.039). Moreover, when compared with controls, more subjects with positive family history were observed in cases (7.73% versus 4.41%, P=0.024).

Genotype and distribution of the CSF-1 and CD-44 between the cases and controls

Distribution of the CSF-1 and CD44 Genotype between the Cases and Controls, and their associations with risk of nephrolithiasis, are shown in **Table 2**. All the probed genotype frequencies of the polymorphisms in the control group conform to the Hardy-Weinberg equilibrium (HWE) (P>0.05). As shown in the **Table 2**, Significant differences can be found in the distribution of the genotype and allele frequencies of rs13347 and rs2050462 between the nephrolithiasis patients and control subjects (P<0.05). For the rs2050462 polymorphism, the frequencies of the CC, CT, and TT genotypes were 43.64%, 51.89%, 4.47% and 39.88%, 49.10%, 11.02%, among nephrolithiasis cases and controls, respectively (P<0.01). The frequencies of the CC, CT, and TT genotypes of rs13347 were 37.80%, 45.88%, and 16.32% among nephrolithiasis cases and 43.49%, 46.49% and 10.02% among controls respectively (P<0.01). For the rs2050462, a significantly decreased nephrolithiasis risk was found in the combined genotype (CA/AA) compared with the CC genotype (OR=0.77, 95% CI=0.63-0.94). Compared with individuals with homozygote CC genotype, those subjects carrying the variant genotypes (CT/TT) of the SNP rs13347 had a significant increased nephrolithiasis risk (OR=1.30, 95% CI=1.09-1.56). No significant difference in genotype and allele distribution of rs8193 and rs333951 were observed between the nephrolithiasis cases and controls (P=1.06 and 1.15, respectively).

Stratification analyses of the two polymorphisms and risk of nephrolithiasis

Further, we evaluated the association between the effect of the two polymorphisms and the

Table 2. Genetic variants in CSF-1 and CD44 associated with the nephrolithiasis risk

Polymorphisms	Cases (n=582)		Controls (n=499)		P[a]	Adjusted OR (95% CI)[b]
	n	%	n	%		
CSF-1 rs2050462						
CC	254	43.64	199	39.88	**0.000**	1.00 (reference)
CA	302	51.89	245	49.10	0.785	0.98 (0.77-1.27)
AA	26	4.47	55	11.02	**0.000**	**0.61 (0.47-0.79)**
CA/AA	328	56.36	300	60.12	0.211	**0.77 (0.63-0.94)**
C	810	69.59	643	64.43	**0.011**	1.00 (reference)
A	354	30.41	355	35.57		**0.80 (0.67-0.96)**
CSF-1 rs333951						
AA	44	7.56	49	9.82	0.301	1.00 (reference)
AG	273	46.91	240	48.10	0.294	1.25 (0.80-1.96)
GG	265	45.53	210	42.08	0.133	1.17 (0.93-1.47)
AG/GG	538	92.44	450	90.18	0.187	1.15 (0.95-1.39)
A	361	31.01	338	33.87	0.157	1.00 (reference)
G	803	68.99	660	66.13		1.13 (0.94-1.36)
CD44 rs13347						
CC	220	37.80	217	43.49	**0.006**	1.00 (reference)
CT	267	45.88	232	46.49	0.334	1.14 (0.88-1.47)
TT	95	16.32	50	10.02	**0.001**	**1.38 (1.13-1.69)**
CT/TT	362	62.20	282	56.51	0.058	**1.30 (1.09-1.56)**
C	707	60.74	666	66.73	**0.004**	1.00 (reference)
T	457	39.26	332	33.27		**1.30 (1.08-1.55)**
CD44 rs8193						
CC	120	20.62	99	19.84	0.343	1.00 (reference)
CT	288	49.48	268	53.71	0.452	0.89 (0.65-1.22)
TT	174	29.90	132	26.45	0.638	1.04 (0.87-1.24)
CT/TT	462	79.38	400	80.16	**0.026**	1.06 (0.89-1.26)
C	528	45.36	466	46.69	0.535	1.00 (reference)
T	636	54.64	532	53.31		1.06 (0.89-1.25)

Bold values indicated significant differences between two groups. [a]Two-sided χ^2 test for either genotype distributions or allele frequencies between the cases and controls. [b]Adjusted for age, BMI, pack-years of smoking, drinking status, tea drinking, hypertension and diabetes in logistic regression model.

risk of nephrolithiasis stratified by age, BMI, sex, smoking status, pack-year of smoking, drinking status, family history of urolithiasis, hypertension and diabetes (shown in **Tables 3**, **4**). The association between CSF-1 rs20504-62 and risk of nephrolithiasis appeared stronger in subgroup of age ≥51 years (P=0.015, OR=0.69, 95% CI=0.52-0.93), BMI >24 (P=0.033, OR=0.74, 95% CI=0.56-0.98), males (P=0.010, OR=0.73, 95% CI=0.57-0.93), drinkers (P=0.045, OR=0.69, 95% CI=0.47-0.99), subjects with negative family history of urolithiasis (P=0.010, OR=0.76, 95% CI=0.62-0.94), subjects without history of hypertension (P=0.037, OR=0.79, 95% CI=0.63-0.99) and subjects without history of diabetes (P=0.008, OR=0.76, 95% CI=0.62-0.93). Similarly, in subgroups of age ≥51 years (P=0.018, OR=1.35, 95% CI=1.05-1.73), BMI ≤24 (P=0.037, OR=1.33, 95% CI=1.02-1.73), males (P=0.049, OR=1.25, 95% CI=1.00-1.56), non-smokers (P=0.017, OR=1.31, 95% CI=1.05-1.63), non-drinkers (P=0.007, OR=1.35, 95% CI=1.09-1.69), subjects with negative family history of urolithiasis (P=0.007, OR=1.29, 95% CI=1.07-1.55), and subjects without history of diabetes (P=0.003, OR=1.32, 95% CI=1.10-1.58), the association between CD44 rs13347 and risk of nephrolithiasis were stronger.

Combined analysis between the two polymorphisms and nephrolithiasis susceptibility

We evaluated the potential interactions of the polymorphisms by combining them based on the number of risk alleles, since all two SNPs (rs2050462 and rs13347) were observed to be associated with nephrolithiasis risk. As shown in **Tables 5**, **6**, in the combined analysis of risk alleles, statistical significant results can be observed between the nephrolithiasis patients and control subjects. Considering that the number of subjects that carried 0 risk alleles was relatively small, the risk alleles were classified into two alleles based on the number of risk alleles. Compared to those carrying 0-1 risk alleles, we found that the risk of nephrolithiasis was significantly increased in subjects that carried 2-4 risk alleles (P=0.027, OR=1.33, 95% CI=1.01-1.75). Furthermore, the association of the combined genotypes and the risk of nephrolithiasis were then evaluated by age, BMI, sex, smoking status, pack-year of smoking, drinking status, Family history of urolithiasis, hypertension and diabetes. As shown in **Table 5**, the effects of combined risk alleles was more obvious in sub-

Table 3. Stratification analysis of CSF-1 rs2050462 and nephrolithiasis risk

Variables	Cases (n=582)				Controls (n=499)				P[a]	Adjusted OR (95% CI)
	CC		CA/AA		CC		CA/AA			
	n	%	n	%	n	%	n	%		
Total	254	43.64	328	56.36	199	39.88	300	60.12	**0.009**	**0.77 (0.63-0.94)**
Age (years)										
<51	123	43.01	163	56.99	102	39.53	156	60.47	0.150	0.82 (0.62-1.07)
≥51	131	44.26	165	55.74	97	40.25	144	59.75	**0.015**	**0.69 (0.52-0.93)**
BMI										
≤24	116	40.56	170	59.44	90	39.82	136	60.18	0.132	0.80 (0.60-1.07)
>24	138	46.62	158	53.38	109	39.93	164	60.07	**0.033**	**0.74 (0.56-0.98)**
Sex										
Male	164	42.93	218	57.07	125	37.76	206	62.24	**0.010**	**0.73 (0.57-0.93)**
Female	90	45.00	110	55.00	74	44.05	94	55.95	0.650	0.92 (0.63-1.33)
Smoking status										
No	176	44.11	223	55.89	138	40.83	200	59.17	0.107	0.82 (0.64-1.04)
Yes	78	42.62	105	57.38	61	37.89	100	62.11	0.063	0.71 (0.49-1.02)
Pack-year of smoking										
0	176	44.11	223	55.89	138	40.83	200	59.17	0.107	0.82 (0.64-1.04)
<20	54	39.71	82	60.29	53	39.55	81	60.45	0.375	0.84 (0.56-1.24)
≥20	24	51.06	23	48.94	8	29.63	19	70.37	0.099	0.39 (0.13-1.19)
Drinking status										
No	185	45.01	226	54.99	138	41.95	191	58.05	0.085	0.81 (0.64-1.02)
Yes	69	40.35	102	59.65	61	35.88	109	64.12	**0.045**	**0.69 (0.47-0.99)**
Family history of urolithiasis										
No	235	43.76	302	56.24	189	39.62	288	60.38	**0.010**	**0.76 (0.62-0.94)**
Yes	19	42.22	26	57.78	10	45.45	12	54.55	0.460	0.70 (0.27-1.81)
Hypertension										
No	199	42.98	264	57.02	158	39.30	244	60.70	**0.037**	**0.79 (0.63-0.99)**
Yes	55	46.22	64	53.78	41	42.27	56	57.73	0.080	0.67 (0.43-1.05)
Diabetes										
No	236	42.83	315	57.17	191	39.38	294	60.62	0.008	0.76 (0.62-0.93)
Yes	18	58.06	13	41.94	8	57.14	6	42.86	0.490	1.75 (0.36-8.55)

Bold values indicated significant differences between two groups. [a]Adjusted for age, BMI, sex, smoking status, pack-year of smoking, drinking status, family history, hypertension and diabetes in logistic regression model.

group of Age ≥51 years (P=0.012, OR=1.66, 95% CI=1.12-2.46), males (P=0.012, OR=1.49, 95% CI=1.06-2.09), subjects with negative family history of urolithiasis (P=0.019, OR=1.38, 95% CI=1.04-1.83), subjects with history of hypertension (P=0.043, OR=1.90, 95% CI=1.03-3.53), and subjects without history of diabetes (P=0.036, OR=1.34, 95% CI=1.02-1.77).

Discussion

In the present study, we investigated the associations of four SNPs in interstitial inflammation-related CSF-1 and CD44 with nephrolithiasis susceptibility in a Chinese population. Finally, it was found that the CSF-1 rs2050462 C allele was a hazardous factor in nephrolithiasis. We also discovered that the subjects carrying the variant genotypes of the SNP rs13347 had a significant increased nephrolithiasis risk compared with those with homozygote genotype. No remarkable difference of genotype frequencies between cases and controls was observed in CD44 rs8193 and CSF-1 rs333951.

Randall's plaques are very common in idiopathic calcium-oxalate nephrolithiasis and are widely known to be a plausible pathogenetic mechanism of common calcium stones [17]. Several

Table 4. Stratification analysis of CD44 rs13347 and nephrolithiasis risk

Variables	Cases (n=582)				Controls (n=499)				P[a]	Adjusted OR (95% CI)[a]
	CC		CT/TT		CC		CT/TT			
	n	%	n	%	n	%	n	%		
Total	220	37.80	362	62.20	217	43.49	282	56.51	**0.004**	**1.30 (1.09-1.56)**
Age (years)										
<51	111	38.81	175	61.19	107	41.47	151	58.53	0.108	1.24 (0.95-1.61)
≥51	109	36.82	187	63.18	110	45.64	131	54.36	**0.018**	**1.35 (1.05-1.73)**
BMI										
≤24	109	38.11	177	61.89	97	42.92	129	57.08	**0.037**	**1.33 (1.02-1.73)**
>24	111	37.50	185	62.50	120	43.96	153	56.04	0.114	1.22 (0.95-1.57)
Sex										
Male	144	37.70	238	62.30	142	42.90	189	57.10	**0.049**	**1.25 (1.00-1.56)**
Female	76	38.00	124	62.00	75	44.64	93	55.36	0.086	1.34 (0.96-1.89)
Smoking status										
No	158	39.60	241	60.40	155	45.86	183	54.14	**0.017**	**1.31 (1.05-1.63)**
Yes	62	33.88	121	66.12	62	38.51	99	61.49	0.101	1.32 (0.95-1.83)
Pack-year of smoking										
0	158	39.60	241	60.40	155	45.86	183	54.14	0.017	1.31 (1.05-1.63)
<20	45	33.09	91	66.91	53	39.55	81	60.45	0.090	1.38 (0.95-2.00)
≥20	17	36.17	30	63.83	9	33.33	18	66.67	0.434	1.42 (0.59-3.40)
Drinking status										
No	159	38.69	252	61.31	152	46.20	177	53.80	**0.007**	**1.35 (1.09-1.69)**
Yes	61	35.67	110	64.33	65	38.24	105	61.76	0.399	1.15 (0.83-1.58)
Family history of urolithiasis										
No	204	37.99	333	62.01	207	43.40	270	56.60	**0.007**	**1.29 (1.07-1.55)**
Yes	16	35.56	29	64.44	10	45.45	12	54.55	0.131	1.98(0.81-4.83)
Hypertension										
No	173	37.37	290	62.64	168	41.79	234	58.21	0.019	1.27 (1.05-1.55)
Yes	47	39.50	72	60.50	49	50.52	48	49.48	0.056	1.51 (0.99-2.33)
Diabetes										
No	210	38.11	341	61.89	213	43.92	272	56.08	**0.003**	**1.32 (1.10-1.58)**
Yes	10	32.26	21	67.74	4	28.57	10	71.43	0.973	0.98 (0.27-3.60)

Bold values indicated significant differences between two groups. [a]Adjusted for age, BMI, sex, smoking status, pack-year of smoking, drinking status, family history, hypertension and diabetes in logistic regression model.

studies have indicated that calcium phosphate may be essential for the attachment of CaOx crystals to epithelial cells [18-21]. Besides, epithelial injury and crystal binding molecules, such as OPN, CD44, and ANX2, may also promote the attachment of CaOx crystals [22-24]. After the crystal attaching to tubular epithelium or endocytosed by tubular epithelium, crystal may further moved into interstitium, which may lead to recruitment of inflammatory cells into interstitium [25]. The recruited inflammatory cells mainly consist of lymphocytes and macrophages [26]. These cells may either remove the crystal through phagocytosis and digestion or release proteases which help ulceration of the interstitial crystals to the papillary surface [27]. Interstitial inflammation was more prevalent among CaOx stone formers (SF) [28]. This may indicate that interstitial inflammation may be an important component part of pathologic processes in CaOx formation. Interstitial inflammation may further leads to stone retention or help ulceration of the interstitial crystals to the papillary surface [25]. Then with supply of calcium, oxalate and other ions by pelvic urine, a stone attached to the papillary surface may form. As we know that CD44 and CSF-1 are related to tissue inflammation and inflammato-

Table 5. Frequency distributions of the number of risk alleles between cases and controls, and their association with nephrolithiasis risk

	Cases		Controls		P^a	Adjusted OR (95% CI)[b]
	n	%	n	%		
Number of risk alleles						
0	14	2.41	33	6.61	0.000	1.00 (reference)
1	123	21.13	114	22.85	**0.006**	**2.99 (1.47-6.11)**
2	231	39.69	218	43.69	**0.005**	**1.54 (1.11-2.15)**
3	174	29.90	111	22.24	**0.000**	**1.57 (1.25-1.98)**
4	40	6.87	23	4.61	**0.000**	**1.47 (1.17-1.85)**
Recombined group						
0-1	137	23.54	147	29.46	**0.027**	1.00 (reference)
2-4	445	76.46	352	70.54		**1.33 (1.01-1.75)**

Bold values indicated significant differences between two groups. [a]Two-sided χ^2 test for either genotype distributions or allele frequencies between the cases and controls. [b]Adjusted for age, BMI, sex, smoking status, pack-year of smoking, drinking status, family history, hypertension and diabetes in logistic regression model.

ry-related Mφ polarization and migration. Thus, it is reasonable to speculate that polymorphisms in CSF-1 and CD44 have an effect on the risk of nephrolithiasis. Our results were indeed consistent with this speculation. The described pathogenetic mechanism of CaOx stones formation could be an explanation of our results to some degrees.

CSF-1, which encodes macrophage colony stimulating factor, is an immune factor for macrophage activation, and its deficiency induces the absence of monocyte differentiation and consecutive depletion of macrophages, osteoclasts, and Kuppfer cells [29]. Mφs can be inflammatory (M1) or anti-inflammatory (M2). CSF-1 mediates polarization to the M2Mφ phenotype [7]. CSF-1 deficiency may result in a smaller population of M2Mφs, which could weaken the suppression of renal crystal deposition. Atsushi Okada et al. were the first to report a suppressive role of CSF-1 signaling in renal crystal formation in mice by influencing the Mφ polarization [30]. We thought these mechanisms were reliable even though some of those results were based on animal models of nephrolithiasis. This was because that this similar mechanism was observed in clinical studies and tissue culture studies of humankind [3]. Based on the described mechanisms above, we speculated that the CSF-1 rs2050462 was related to nephrolithiasis risk via influencing the potential activity of CSF-1. To our knowledge, this is the first report of an association of CSF-1 polymorphisms with susceptibility of nephrolithiasis.

A previous Genome-wide association studies (GWAS) study examined a total of 52 SNPs located in CD44 gene region [31]. Allele frequencies of 12 SNPs (not including rs13347) showed significant differences ($P<0.05$) between the kidney stone disease patient and control groups in that GWAS study. Similar to CSF-1, CD44 is also related to tissue inflammation and inflammatory-related Mφ migration. However, the difference is that CD44 could inversely induce renal crystal formation by inhibiting M2 phagocytic activity [30]. Besides, the expression of OPN and CD44 by tubular cells seems to play a role in retention of crystals in a rat model [24]. CD44 can interact with OPN, the major component in the urinary stone matrix and play an important role in CaOx crystal binding to renal epithelial cells. Therefore, it was rational that the rs13347 in CD44 can increase the risk of nephrolithiasis. Our results could further confirm the association between polymorphisms of CD44 and the risk of nephrolithiasis, and may provide new insight into function of polymorphisms of CD44 in nephrolithiasis.

Through stratification analyses, we probed that the association between CSF-1 rs2050462 and CD44 rs13347 and nephrolithiasis risk was more prominent in subgroups of age ≥51 years, supported by several researches which announced that DNA damage and mutation were increasing with age [32, 33]. When shifting our attention to the BMI subgroup, divergence has been observed between two SNPs. For CD44 rs13347, subjects with a lower BMI have a stronger association with risk of nephrolithiasis. As for CSF-1 rs2050462, subjects with a higher BMI got more protection. Given the subtle effect of metabolic syndrome on kidney stone disease [34, 35], the results above can be explained by the different relationship between the two SNPs and metabolic issue. We also found that the association between nephrolithiasis risk and two SNPs was stronger in males. A demographics study showed that gender could influence excretion of critical urinary factors related to kidney stone risk, and should be taken into account when evaluating kidney stone patients [36, 37]. The association was

Table 6. Stratification analysis of number of risk alleles and nephrolithiasis risk

Variables	Cases (n=582)				Controls (n=499)				P[a]	Adjusted OR (95% CI)[b]
	Number of risk alleles				Number of risk alleles					
	0-1		2-4		0-1		2-4			
	n	%	n	%	n	%	n	%		
Total	137	23.54	445	76.46	147	29.46	352	70.54	0.027	1.33 (1.01-1.75)
Age (years)										
<51	73	25.52	213	74.48	72	27.91	186	72.09	0.530	1.12 (0.76-1.64)
≥51	64	21.62	232	78.38	75	31.12	166	68.88	**0.012**	**1.66 (1.12-2.46)**
BMI										
≤24	72	25.17	214	74.83	65	28.76	161	71.24	0.363	1.13 (0.76-1.69)
>24	65	21.96	231	78.04	82	30.04	191	69.96	0.028	1.47 (1.00-2.15)[c]
Sex										
Male	86	22.51	296	77.49	102	30.82	229	69.18	**0.012**	**1.49 (1.06-2.09)**
Female	51	25.50	149	74.50	45	26.79	123	73.21	1.780	1.00 (0.61-1.65)
Smoking status										
No	98	24.56	301	75.44	102	30.18	236	69.82	0.088	1.29 (0.93-1.79)
Yes	39	21.31	144	78,69	45	27.95	116	72.05	0.153	1.44 (0.85-2.43)
Pack-year of smoking										
0	98	24.56	301	75.44	102	30.18	236	69.82	0.088	1.29 (0.93-1.79)
<20	30	22.06	106	77.94	37	27.61	97	72.39	0.291	1.44 (0.81-2.56)
≥20	9	19.15	38	80.85	8	29.63	19	70.37	0.302	1.34 (0.36-5.03)
Drinking status										
No	101	24.57	310	75.43	94	28.57	235	71.43	0.220	1.20 (0.86-1.67)
Yes	36	21.05	135	78.95	53	31.18	117	68.82	0.033	1.61 (0.97-2.67)
Family history of urolithiasis										
No	125	23.28	412	76.72	142	29.77	335	70.23	**0.019**	**1.38 (1.04-1.83)**
Yes	12	26.67	33	73.33	5	22.73	17	77.27	0.728	1.50 (0.38-5.95)
Hypertension										
No	109	23.54	354	76.46	112	27.86	290	72.14	0.146	1.24 (0.91-1.69)
Yes	28	23.53	91	76.47	35	36.08	62	63.92	**0.043**	**1.90 (1.03-3.53)**
Diabetes										
No	134	24.32	417	75.68	146	30.10	339	69.60	**0.036**	**1.34 (1.02-1.77)**
Yes	3	9.68	28	90.32	1	7.14	13	92.86	0.782	1.70 (0.11-27.22)

Bold values indicated significant differences between two groups. [a]Two-sided χ^2 test for either genotype distributions or allele frequencies between the cases and controls. [b]Adjusted for age, BMI, sex, smoking status, pack-year of smoking, drinking status, family history of urolithiasis, hypertension and diabetes in logistic regression model. [c]Actual Adjusted OR (95% CI): 1.47 (0.998-2.156).

stronger in non-smokers and non-drinkers of CD44 rs13347. It might be because environmental factors were more predominant than genetic cause of this polymorphism [38, 39]. In addition, subjects without history of diabetes and hypertension bear less risk in stratification analyses of CSF-1 rs2050462. According to a former study, diabetes mellitus, hypertension may add to the possibility of stone formation [40]. Interestingly, the influence of two SNPs was more prominent in subjects with negative family history of urolithiasis. Heredity of urolithiasis susceptibility may play a special role in prevalence of nephrolithiasis risk [41, 42], which may partly explain the difference between two subgroups.

Macrophages and multinucleated giant cells may play a special role in Crystal retention. CD44 and CSF-1 were all related to tissue inflammation and inflammatory-related Mφ polarization and migration. Moreover, CSF-1

may have a subtle relationship with the expression of OPN and CD44. Based on an animal experiment, CSF-1 deficiency could significantly increase the expression of OPN and CD44 at both the mRNA and protein levels [29]. In view of the above-mentioned facts, analysis of combined alleles was done to estimate genetic susceptibility for candidate genes comprehensively. Statistical significant results were observed between the nephrolithiasis patients and control subjects in the combined analysis of risk alleles. In theory, subjects with joint genotypes containing 4 risk alleles should take more risk of nephrolithiasis than those carrying less than four alleles. However, individuals with 1 risk alleles inversely got a maximal Adjusted OR. These significant results may not reliable because the number of subjects that carried 0 risk allele was relatively small. So we further classified the risk alleles into two groups based on the number of risk alleles. After recombination, a significant higher risk of developing nephrolithiasis was observed in subjects with joint genotypes containing 2-4 risk alleles than those whose joint genotypes containing 0-1 risk alleles. Took the subtle relationship of CSF-1 and CD44 into consideration, this result may provide a more comprehensive prediction of genetic susceptibility for subjects carrying no less than 1 risk alleles. It is universally accepted that genetic inheritance and environmental exposure may cause the incidence of stones by a synergistic effect. So stratification analyses were done to further probe the association between the number of risk alleles and nephrolithiasis risk. Through subgroup analysis, we thought genetic effects on nephrolithiasis susceptibility may be interfered by age, male, physical condition exposure.

Overall, this study proved interstitial inflammation-related genetic variants in CD44 and CSF-1 were essential to risk of nephrolithiasis. Our findings may be helpful to clinical diagnosis and prediction of nephrolithiasis. However, some limitations of this study should be noted. Firstly, to apply new markers in the clinical practice needs complicated steps and diverse validation analyses. More studies on expression and activity of inflammation-related genetic variants in CD44 and CSF-1 in subjects with different genotypes are indispensible. Secondly, our findings merit further evaluation in larger series of nephrolithiasis patients from different ethnicities and regions, taking into account that nephrolithiasis is a heterogeneous illness with multiple confounders. Last but not the least, lack of detailed data of prognosis and other risk factors, such as recurrence of nephrolithiasis, dietary factor, laboratory data and clinical characteristic from patients limits further in-depth investigation.

Conclusion

To sum up, this current research firstly provided evidences to certify the association between interstitial inflammation-related genetic variants in CD44 and CSF-1 and nephrolithiasis susceptibility. Especially, the combined risk alleles may be promising novel predictors to forecast development of nephrolithiasis. Our investigation can also give the evidence for the special role macrophages and multinucleated giant cells played in crystal retention or accumulation with mediation of CSF-1 and CD44 in the kidney.

Acknowledgements

This paper is supported by National Natural Science Foundation of China [grant number 81171963, 81201998, 81201571]. This work was supported by the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), by the Jiangsu Provincial Special Program of Medical Science (BL2012027), by the Program for Development of Innovative Research Team in the First Affiliated Hospital of Nanjing Medical University, by the National Natural Science Foundation of China [grant number 81171963, 81201998, 81201571].

Disclosure of conflict of interest

None.

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