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ORIGINAL PAPER

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Interleukin-1β gene and receptor antagonist gene polymorphisms in patients with calcium oxalate stones

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Abstract Interleukin-1 (IL-1) might play a role in the process of bone loss and hypercalciuria and is therefore considered to be involved in the formation of urinary stones. The aim of this study is to test whether the IL-1β promoter region, exon 5 region and IL-1 receptor antagonist gene intron 2 polymorphisms could be genetic markers for the susceptibility to the formation of urinary stones. A control group of 152 healthy people and a group of 105 patients with recurrent calcium oxalate stone were examined in this study. Polymerase chain reaction (PCR) analyzed the variable number tandem repeats at intron 2 of the IL-1Ra gene for the polymorphisms. PCR-based restriction analysis was done for the IL-1β gene polymorphisms of the promoter region and exon 5 by the endonucleases *Ava* I and *Taq* I, respectively. The polymorphisms studied in the IL-1β genes did not reveal a strong association with calcium oxalate stone disease when compared with the control group (promoter region by chi-square test, $P = 0.627$; exon 5 region by Fisher's exact test, $P = 0.403$). Only two frequent alleles of the IL-1Ra gene corresponding to one and two copies of an 86-bp sequence repeat were identified by PCR. The result revealed significant differences between control individuals and stone patients ($P < 0.01$, Fisher's exact test). In addition, the frequency of the type I allele in the stone group (99.0%) was higher than in the control group (94.0%). The odds ratio for the type I allele of the IL-1Ra gene in calcium oxalate stone disease is 6.041 (95% CI: 1.683∼21.687). There is an association between urolithiasis and polymorphism in the IL-1Ra gene. No significant difference was found when dividing the stone patients into groups with normocalciuria and hypercalciuria in relation to these genetic polymorphisms. Further studies of the type I allele of the IL-1Ra gene are worthwhile because of its correlation with stone disease. In our study, neither the IL-1β promoter region nor the exon 5 polymorphisms were significantly different when comparing control subjects and calcium oxalate stone patients.

Keywords Interleukin-1β · Interleukin-1 receptor antagonist gene · Urolithiasis · Single nucleotide polymorphisms (SNPs) · Calcium oxalate

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Introduction

Calcium oxalate stone disease is complex and multifactorial. Genetic markers of this disease could be clinically useful for identifying patients at risk and for preventing the occurrence of urolithiasis. Recently, single nucleotide polymorphisms (SNPs) were used as a tool for mapping the complex disease genes [7]. However, identifying a common genetic marker associated with the susceptibility to urinary stone disease presents an important challenge. Regardless of the difficulties, genes for calcium metabolism, such as the vitamin D receptor gene polymorphism, have been proposed as candidates in this search for an association [5]. Therefore, SNPs could be used to search for possible genetic markers of urolithiasis.

Cytokines are present in urine and several researchers have reported their association with stone disease [11, 15, 16]. One such cytokine, interleukin-1 (IL-1), is a

potent proinflammatory agent, playing a central role in joint inflammation and destruction. In addition, it may induce bone resorption through stimulation of IL-6 and induce osteoclast formation [10]. Therefore, the IL-1 gene has been used as a genetic marker for the prediction of bone density in menopausal women [6]. An excessive amount of IL-1 mRNA transcription was noted in hypercalciuric patients, indicating that IL-1 may be a candidate for the SNPs study of calcium oxalate stone disease [16]. However, to our knowledge, a survey of the relationship between the IL-1 gene and calcium oxalate stone disease has yet to be reported.

Polymorphisms of the Il-1β promoter region, exon 5 and receptor antagonist (IL-1Ra) exon2 gene, have been used to screen the relationship between the occurrence and severity of rheumatoid arthritis and osteoporosis [3, 6]. We therefore tried to use polymerase chain reaction (PCR) analysis for the investigation of the distribution between the control group and stone patients. To test whether these polymorphisms could act as markers for susceptibility to calcium oxalate stone disease, we compared allelic frequencies in a normal, control population with recurrent calcium oxalate disease patients by screening the IL-1 geneβ promoter region, the exon 5 region and IL-1Ra gene intron 2 polymorphisms.

Patients and methods

Patient selection

A total of 152 patients (117 males and 35 females) aged between 23 and 76 years (average age: 44.62 ± 12.05 years) with recurrent calcium oxalate stone were enrolled in this study. There were 32 patients with hypercalciuria as defined by 24 h urine calcium of more than 300 mg in males and 250 mg in females on a random diet (21 male and 11 female patients). The remaining patients revealed a normal urine calcium level and were categorized as normocalciuria (96 male and 24 female patients). There were 60 males and 45 females in the control group (age range from 40 to 87 years with an average of 53.02 ± 10.08 years). Serial blood and urine biochemistry tests were undertaken to exclude possible hypercalcemia, hyperuricemia, or hyperuricosuria. Patients who showed symptoms of urinary tract infections during the period of stone treatment were excluded. Stone composition was verified by infrared spectroscopy and revealed either calcium oxalate monohydrate, dihydrate, or a combination of the two. A control group was drawn up of 105 healthy volunteers over the age of 40 who had no history of familial stone disease or renal calcification following renal ultrasonography tests and routine urinary microscopic hematuria. Informed consent was obtained from both groups that participated in this study. The genomic DNA was prepared from peripheral blood using a Genomaker reagent kit (Blossom, Taiwan).

Polymerase chain reaction

PCR was used to identify the genotypes of all of the IL-1 related genes. For determining the polymorphisms a total volume of 50 µl, containing genomic DNA(2–6 *p*mole of each primer); 1× Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, USA), was used for PCR. The primers for the IL-1β promoter region, exon 5 and IL-1Ra gene polymorphisms are listed in Table 1, according to the report by Cantagrel and co-workers [3]. PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Perkin Elmer). The cycling condition is given in Table 1.

For the IL-1Ra intron 2, 10 µl of the products were loaded into a 3% agarose gel containing ethidium bromide for electrophoresis, and each allele was recognized according to its size. The 86-bp variable number tandem repeat (VNTR) of the IL-1Ra gene was classified as types I for 410-bp, II for 240-bp, III for 500-bp, IV for 325-bp, and V for the 595-bp allele. The IL-1β promoter polymorphism at position 511 was analyzed by PCR amplification followed by restriction analysis using *Ava* I (New England Biolabs, Beverly, USA) digestion. The C allele at position 511 was categorized as "C" and showed as 190-bp and 114-bp after agarose electrophoresis. The "T" allele was 304-bp and was encoded at position 511. The region containing the polymorphic site within exon 5 of the IL-1β gene was amplified and then digested by *Taq* I (New England Biolabs). Class "E1" was 135-bp and 114-bp and "E2" was 249-bp as shown by electrophoresis.

For statistical analysis, the allelic frequency distributions of these polymorphisms in the control and stone patient groups were compared using the chi-square test. When an assumption of the chi-square test was violated (i.e., when one cell had an expected count of < 1, or $> 20\%$ of the cells had an expected count of < 5), the Fisher's exact test was used. Odds ratios (OR) with 95% confidence intervals (CI) were determined for the disease susceptibility of patients with specific alleles in the IL-1Ra gene. Results are considered statistically significantly when the probability of the findings occurring by chance is less than 5% ($P < 0.05$).

Results

Using Fisher's exact test, we did not find a significant difference in allele frequencies between stone patients and control at the IL-1β gene exon 5 polymorphism (Table 2, $P = 0.403$). No difference between patients and

Table 1 Sequences of the amplification primers in the 5′ to 3′ orientation (*PCR*, polymerase chain reaction, *VNTR* variable number tandem repeats, *U* upstream primers, *D* downstream primers)

Set	Position	Primer and PCR conditions	PCR product (bp)	Restriction site
Il-1β promoter	511 C/T	U 5′-TGGCATTGATCTGGTTCATC-3′ D 5′-GTTTAGGAATCTGGACCAGA-3′ 95 °CX30″, 55 °CX30″, and 72 °CX30″	190 + 114 or 304	*Ava* I
IL-1β exon 5	Exon 5	U 5′-GTTGTCATCAGACTTTGACC-3′ D 5′-TTCAGTTCATATGGACCAGA-3′ 95 °CX30″, 55 °CX30″, and 72 °CX30″	135 + 114 or 249	*Taq* I
LI-1Ra	Intron 2	U 5′-CTCAGCAACACTCCTAT-3′ D 5′-TCCTGGTCTGCAGGTAA-3′ 95 °CX30″, 58 °CX30″, and 72 °CX30″	I:410 II:240	VNTR

Table 2 Distribution of interleukin-1β receptor promoter gene polymorphism between the control subjects and the calcium oxalate stone patients. df = 2 in Chi-square test

	C/C		C/T		T/T		Total		χ^2	*P*-value
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)		
Control	28	26.7	50	47.6	27	25.7	105	100.0		
Stone patient	49	32.2	66	43.4	37	24.3	152	100.0	0.933	0.627
Total	77	32.2	116	43.4	64	24.3	257	100.0		
Normocalciuria	39	32.5	53	44.2	28	23.3	120	100.0		
Hypercalciuria	10	31.3	13	40.6	9	28.1	32	100.0	0.323	0.850
							100.0	100.0		

control individuals was found at the Il-1β promoter region polymorphism (Table 3, $P = 0.627$).

The bands on the gel revealed variable number tandem repeat (VNTR) allele type I homozygotes, type II homozygotes, and type I/II heterozygotes. There were no type III, IV and V alleles found in this study in either the control population or the patient groups. The frequencies of the genotypes in the stone group and control group are shown in Table 4. Using Fisher's exact test, the distribution of IL-1Ra gene polymorphism was compared. This showed significant differences between the control group and the stone patient group ($P < 0.01$). The distribution of type I homozygotes in the control group was 89.3% compared with 97.9% in the stone group. No type II homozygote was found in the stone group. The allelic distribution of the I/II polymorphism at the IL-1Ra gene in control subjects showed type I allele at 94% and type II allele at 6%. Whereas the stone patients showed type I allele at 99% and type II allele at 1%. The odds ratio for the risk of type I allele in stone patient is 6.041 with 95% CI of 1.683 to 21.687. Overall, there was no statistical difference between the groups of normocalciuria and hypercalciuria in stone patients for these genetic polymorphisms.

Discussion

Cytokines are known to be involved in the formation of calcium oxalate stone disease, although no cytokine polymorphism has been studied. A great amount of the genetic background of calcium oxalate disease is still unidentified, and only a few gene polymorphisms have been studied [5, 12]. We chose to focus on cytokines as candidate genes because they have several proteins that are key components in the pathogenesis of many diseases. However, in our control group, only the allelic frequencies of the IL-1β gene promoter region were similar to those previously reported in other controls. There was a relatively low incidence of II allele in IL-1Ra and E2 allele in IL-1β gene exon 5 in the control group. Ethnic differences could be considered in interpreting this result.

Table 3 Distribution of interleukin-1β receptor exon 5 gene polymorphism between the control subjects and the calcium oxalate stone patients

	E1/E1		E1/E2		E2/E2		Total		*P*-value
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	
Control	102	97.1	3	2.9	0	0	105	100.0	
Stone patient	149	98.7	2	1.3	0	0	151	100.0	0.403
Total	251	98.7	5	1.3	0	0	256	100.0	
Normocalciuria	117	98.3	2	1.7	0	0	119	100.0	
Hypercalciuria	32	32 (100.0	0	0.0	0	0	32	100.0	1.0

Fisher's exact test

Table 4 Distribution of interleukin-1 receptor antagonist (IL-1Ra) gene polymorphism between the control subjects and the calcium oxalate stone patients. *$P < 0.01$, Fisher's exact test

	I		I and II		II		Total		*P*-value
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	
Control	92	89.32	10	9.70	1	0.97	103	100.00	
Stone patient	145	98.0	3	2.0	0	0.0	148	100.0	0.005*
Total	237	97.97	13	2.02	1	0.0	251	100.00	
Normocalciuria	114	98.3	2	1.7	0	0.0	116	100.0	
Hypercalciuria	31	96.9	1	3.1	0	0.0	32	100.0	0.521
Total	145	98.0	3	2.0	0	0.0	148	100.0	

Although most studies have focused on the vitamin D receptor gene [5, 12], other genes alone or in combination could be involved in modulating calcium oxalate metabolism. There was a significant association between stone and IL-1Ra alleles in this study. Because IL-1Ra is a hormone receptor coupled with a G protein, a single change in the intracellular domain may occasionally cause disease. It is considered possible that these two allelic variants of the IL-1Ra gene may have an influence over the variation of intracellular signal pathways and may be associated with some diseases [9].

IL-1Ra is an important regulator of inflammation. IL-1Ra is IL-1's natural competitive inhibitor, occupying IL-1 cell surface receptors without triggering signal transduction [1]. The type II IL-1Ra allele has been previously found in association with a variety of autoimmune diseases such as alopecia areata, systemic lupus erythematosus, and ulcerative colitis [2, 8, 14]. However, instead of type II, the type I IL-1Ra intron 2 allele did influence the susceptibility to stone disease in our study. The allelic distribution of IL-1Ra gene intron 2 was different in Taiwanese patients compared to white French patients in relation to rheumatoid arthritis [3]. In our group, there were no type III, IV or V alleles in the control group or the stone patients. No type II allele was shown in stone patients, indicating an increase in susceptibility to urolithiasis. Therefore, IL-1Ra involved in the formation of stone formation may differ from that involved in autoimmune disease and may function through a complex pathway.

IL-1 is involved in a wide spectrum of biological activities such as increased body temperature and protein and energy mobilization in the acute phase response [4]. Although it has been proposed that the IL-1 gene contributes to regulating the level of IL-1β production, we did not find the same association with stone disease. Langdahl and co-workers reported a similar finding with the association of osteoporotic fracture [6]. The type I allele of IL-1Ra was significantly associated with osteoporosis, whereas three polymorphism sites of the IL-1β genes were not associated with osteoporotic fractures or alterations in bone mass or bone turnover. This might be caused by an inappropriate choice of the polymorphism region in IL-1 making the statistical difference insignificant. However, because the relationship between IL-1Ra and stone disease is not only involved in an inflammatory pathway, the relationship of IL-1 urolithiasis should be further studied. The genes for IL-1 are located on chromosome 2, in close linkage with the IL-1Ra gene [13]. Linkage disequilibrium of the genes during recombination may be one of the causes.

Patients with urolithiasis were mostly diagnosed after the development of symptoms due to a lack of predictive markers for the disease. Therefore, a reliable marker for urolithiasis could lead to earlier diagnosis and treatment and have a significant impact on improved patient care as well as effective control of health care costs. The data indicate that the IL-1Ra intron 2 polymorphism might be a candidate genetic marker to screen for the association of calcium oxalate stone. The type I allele was prominent in the stone group. Individuals with the type I allele in the IL-1Ra gene intron 2 were expected to have a high risk of calcium oxalate stone disease. In addition of this candidate gene, all of the cytokine genes should be studied as network in further investigations.

References

1. Arend W (1991) Interleukin 1 receptor antagonist: a new member of the interleukin 1 family. J Clin Invest 5:1445
2. Blakemore AIF, Tarlow JK, Cork MJ, Gordon C, Emery P, Duff GW (1994) Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. Arthritis Rheum 37:1380
3. Cantagrel A, Navaux F, Loubet-Lescoulie P, Nourhashemi F, Enault G, Abbal M, Constantin A, Laroche M, Mazieres B (1999) Interleukin-1beta, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: relationship to occurrence and severity of rheumatoid arthritis. Arthritis Rheum 42:1093
4. Fearon DT, Locksley RM (1996) The instructive role of innate immunity in the acquired immune response. Science 272:50
5. Jackman SV, Kibel AS, Ovuworie CA, Moore RG, Kavoussi LR, Jarrett TW (1999) Familial calcium stone disease. TaqI polymorphism and the vitamin D receptor. J Endourol 13:313
6. Langdahl BL, Lokke E, Carstens M, Stenkjaer LL, Eriksen EF (2000) Osteoporotic fractures are associated with an 86-base pair repeat polymorphism in the interleukin-1-receptor antagonist gene but not with polymorphisms in the interleukin-1beta gene. J Bone Miner Res 15:402
7. Lorder N (1999) Genetic variations can point the way to disease gene. Nature 401:734
8. Mansfield JC, Holden H, Tarlow JK, di Giovine FS, McDowell TL, Wilson AG, Holdsworth CD, Duff GW (1994) Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. Gastroenterology 106:637
9. Nakamura E, Takahashi S, Ishikawa M, Okabe S (1999) Inhibitory effect of macrophage-derived factors on the recovery of wounds induced in rat gastric epithelial monolayers. Biochem Pharmacol 58:1221
10. Passeri G, Girasole G, Jilka RL, Manolagas SC (1993) Increased interleukin-6 production by murine bone marrow and bone cells after estrogen withdrawal. Endocrinology 133:822
11. Rhee E, Santiago L, Park E, Lad P, Bellman GC (1998) Urinary IL-6 is elevated in patients with urolithiasis. J Urol 160:2284
12. Ruggiero M, Pacini S, Amato M, Aterini S, Chiarugi V (1999) Association between vitamin D receptor gene polymorphism and nephrolithiasis. Miner Electrolyte Metab 25:185
13. Steinkasserer A, Spurr NK, Cox S, Jeggo P, Sim RB (1992) The human IL-1 receptor antagonist gene (IL1RN) maps to chromosome 2q14-q21, in the region of the IL-1alpha and IL-1beta loci. Genomics 13:654
14. Tarlow JK, Clay FE, Cork MJ, Blakemore AIF, McDonagh AJG, Messenger AG, Duff GV (1994) Severity of alopecia areata is associated with a polymorphism in the interleukin-1 receptor antagonist gene. J Invest Dermatol 103:387
15. Tsai CY, Wu TH, Yu CL, Lu JY, Tsai YY (2000) Increased excretions of beta2-microglobulin, IL-6, and IL-8 and decreased excretion of Tamm-Horsfall glycoprotein in urine of patients with active lupus nephritis. Nephron 85:207
16. Weisinger JR, Alonzo E, Bellorin-Font E, Blasini AM, Rodriguez MA, Paz-Martinez V, Martins R (1996) Possible role of cytokines on the bone mineral loss in idiopathic hypercalciuria. Kidney Int 49:244