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Correlation between the development of calcium oxalate stones and polymorphisms in the fibronectin gene in the Uighur population of the Xinjiang region of China

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ABSTRACT. Here, we have investigated the correlation between calcium oxalate stone formation and *Fn* gene polymorphisms in urinary calculi patients among the Uighur population (Xinjiang region). In this case control study, genomic DNA extracted from the peripheral blood of 129 patients with calcium oxalate stones (patient group) and 94 normal people (control group) was used to genotype polymorphisms in the rs6725958, rs10202709, and rs35343655 sites of the *Fn* gene by polymerase chain reaction-restriction fragment length polymorphism. Subsequently, the association between different genotypes and susceptibility to calcium oxalate stone formation was compared among the patient and control groups. Single nucleotide polymorphisms (SNPs) were detected in the rs6725958, rs10202709, and rs35343655 sites of the *Fn* gene among the patient and control groups. The genotype distributions of the three

loci complied with the Hardy-Weinberg equilibrium. The results of allele frequencies of the patient/control group for polymorphisms in the rs6725958 site of the *Fn* gene were C = 179 (69.92%)/119 (63.30%) and A = 77 (30.08%)/69 (36.70%), in the rs10202709 site were C = 245 (95.70%)/176 (93.63%) and T = 11 (4.30%)/12 (6.38%), and in the rs35343655 site of the *Fn* gene were A = 139 (54.30%)/87 (46.28%) and G = 117 (45.70%)/101 (53.72%). We observed no significant differences between the three SNPs and development of calcium oxalate stones. Polymorphisms in rs6725958, rs10202709, and rs35343655 of the *Fn* gene had no obvious effect on the susceptibility to the development of calcium oxalate stones in the Uighur population, residing in the Xinjiang region of China.

Keywords: Calcium oxalate stones; Fibronectin; Gene polymorphism; Uighur

INTRODUCTION

Urinary calculi is a common disease affecting humans worldwide, and is one of the three major diseases affecting the urinary system. Approximately 10% of the population suffers from urinary calculi, with 70% of these cases exhibiting the development of calcium oxalate stones. The latter represents the idiopathic form of the disease with no clear genetic background and relatively systemic diseases, clinically (Deng et al., 2009; Worcester and Coe, 2010). The recurrence rate of urinary calculi remains high despite the considerable progress in the design of therapeutic strategies for the same (Brikowski et al., 2008). The exact mechanism of urinary calculi remains unclear because of the complicated etiology related to living habits, natural environment, and inheritance. In addition, there are significant regional and racial differences in the etiology (Soucie et al., 1994). Polymorphisms in the ficronectin gene (*Fn*) were analyzed and compared along three sites (rs6725958, rs10202709, and rs35343655) between patients displaying the presence of calcium oxalate stones and healthy people, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). In addition, we studied the correlation between the development of calcium oxalate stones and the *Fn* gene polymorphisms in the Uighur population of the Xinjiang area in China.

MATERIAL AND METHODS

Study subjects

This retrospective study analyzed information regarding 129 unrelated (by blood) Uighur patients (average age 43 ± 10) with urinary calculi, diagnosed based on clinical symptoms, and X-ray plain film and B-mode analyses. All patients were operated in the hospital, or received extracorporeal shock-wave lithotripsy (ESWL) treatment; the samples were determined to be composed of calcium oxalate stones. The control group was comprised of 94 healthy volunteers (average age 46 ± 9) without a history of urinary calculi. Patient and volunteer information was obtained from the Department of Urinary Surgery of the First Teaching Hospital of Xinjiang Medical University and the Second Affiliated Hospital of Xinjiang Medical University. There were no significant differences in the sex and age between the case and control groups (P > 0.05).

Written approval for the study was obtained from the institutional review board of the First Teaching Hospital of Xinjiang Medical University and the Second Affiliated Hospital of Xinjiang Medical University. Informed consent was obtained from all patients and controls.

Methods

Genomic DNA extraction

Blood samples (2 mL) were drawn from the antecubital vein of the members of the case and control groups; the samples were stored in ethylene diamine tetraacetic acid (EDTA) at -20°C. DNA was extracted using the blood genomic DNA extraction kit (K5017500; BioChain Science & Technology, Inc., Beijing, China). The integrity of the genomic DNA was analyzed by agarose gel electrophoresis.

PCR amplification

The PCR primers were obtained from BBI Life Sciences Corporation, 01035.HK. The primer sequences used for the amplification of specific sites on the *Fn* gene are listed in Table 1. Specific DNA sequences were amplified by PCR *in vitro* in a 15 µL reaction system (composition of the system is summarized in Table 2). The reaction conditions for PCR were set as described in Table 3.

Table 1. PCR primer sequences.

Site	Primer sequence
rs6725958	F: 5'-CTCAGGACTTGGATGGTGTAGA-3'
	R: 5'-TCATTTCCCAATAAAAGTACACTG-3'
rs10202709	F: 5'-CAGTCCCAGATCATGGAGTCT-3'
	R: 5'-GTACCATGTTACTTGTGGAATAGAG-3'
rs35343655	F: 5'-ACTGAAGTGCTCGGGATGAT-3'
	R: 5'-CAGGAACGAAATGTTGGATG-3'

F = forward primer; R = reverse primer.

Table 2. PCR amplification system (15 µL reaction system).

Composition	Volume (µL)
Ultrapure water	11.0
2X PCR Mix	3.1
Primer: forward	0.2
reverse	0.2
Template DNA	0.5
Total volume	15.0

dNTP (10 mM).

Table 3. PCR amplification conditions.

Step	Temperature (°C)	Time	Cycle number
Pre-degeneration	95	5 min	
Degeneration	95	30 s	40 cycles
Annealing	68	345 s	
Extension	72	60 s	
Terminal extension	72	6 min	1 cycle
Storage	4	→∞	

Statistical analysis

The polymorphism allele frequencies were found to be consistent with Hardy-Weinberg equilibrium. Genotypes, differences in genotype frequency distribution, and the correlation of specific genotypes with the occurrence and development of calcium oxalate stones were analyzed by the chi-squared test. The measurement data was expressed as mean ± standard deviation (SD). All data was analyzed on the SPSS software platform (v.17.0; SPSS Inc., Chicago, IL, USA). P value < 0.05 was considered to be statistically significant.

RESULTS

Genotype detection

The PCR analyses revealed the presence of polymorphisms at the specific sites rs6725958 (mutant A/A (256 bp), wild type C/C (171, 85 bp), and heterozygote A/C (256, 171, 85 bp) phenotypes were obtained; Figure 1), and rs10202709 (mutant T/T (202 bp), wild type C/C (134 and 68 bp), and heterozygote C/T (202, 134, 68 bp) phenotypes; Figure. 2) following digestion with the restriction enzyme *Hae*III, and at the site rs35343655 (mutant G/G (139, 97 bp), wild type A/A (236 bp), and heterozygote A/G (236, 139, and 97 bp; Figure 3) following digestion with the restriction enzyme *Msp*I.

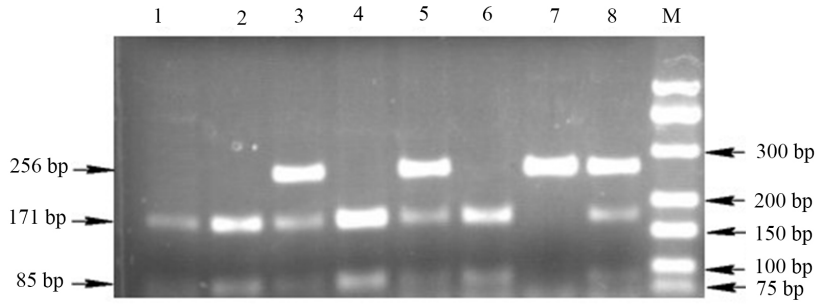

Figure 1. Genotyping of the rs 6725958 polymorphism site in the *Fn* gene.

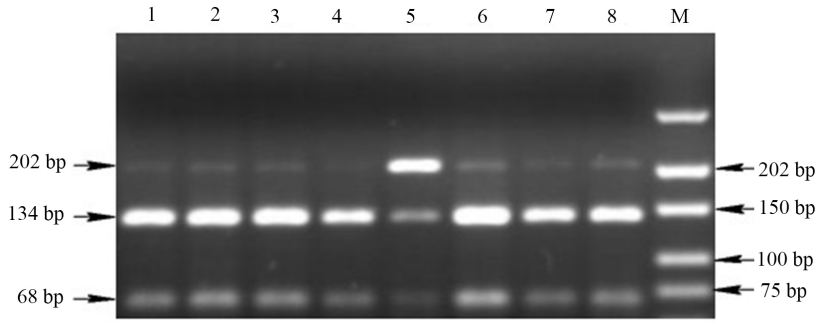

Figure 2. Genotyping of the rs 10202709 polymorphism site in the *Fn* gene.

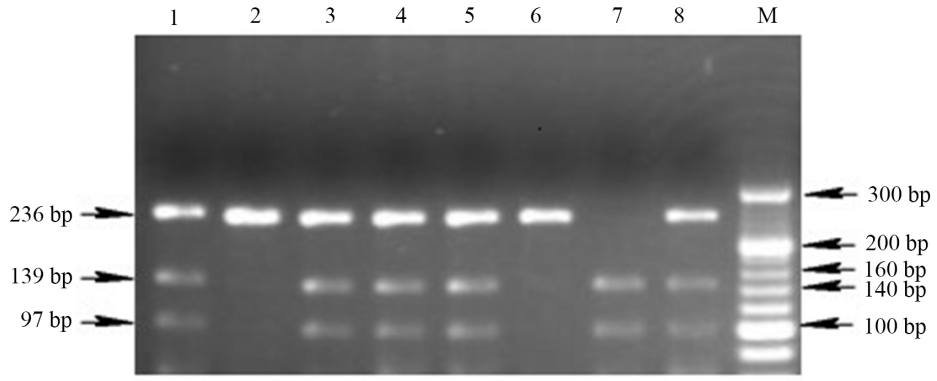

Figure 3. Genotyping of the rs 35343655 polymorphism site in the *Fn* gene.

Comparison of genotype and allele frequency

The distribution of genotype and gene frequency of the SNPs rs6725958, rs10202709, and rs35343655 was consistent with the Hardy-Weinberg equilibrium. No significant differences were observed between the genotype and allele frequencies of cases and controls (Tables 4, 5, and 6).

Table 4. Phenotypes resulting from SNPs in the polymorphism site rs6725958 of the *Fn* gene.

Subjects	Cases (N = 128)	Control (N = 94)	OR	95%CI	P value
Genotype (%)					
C/C	64 (50.00)	36 (38.30)	1	-	
A/C	51 (39.84)	47 (50.00)	1.638	0.927-2.894	0.088
A/A	13 (10.16)	11 (11.70)	1.504	0.611-3.703	0.373
A/C+A/A	64 (50)	58 (61.70)	1.611	0.938-2.768	0.083
Allele frequency (%)			1	-	
C	179 (69.92)	119 (63.30)			
A	77 (30.08)	69 (6.70)	1.348	0.904-2.009	0.142

OR = odd's ratio; CI = confidence interval.

Table 5. Phenotypes resulting from SNPs in the polymorphism site rs10202709 of the *Fn* gene.

Subjects	Cases (N = 128)	Control (N = 94)	OR	95%CI	P value
Genotype (%)					
C/C	117 (91.41)	82 (87.23)	1	-	
C/T	11 (8.59)	12 (12.77)	1.557	0.655-3.699	0.313
T/T	0 (0)	0 (0)	-	0	
C/T+T/T	11 (8.59)	12 (12.77)	1.557	0.655-3.699	0.313
Allele frequency (%)					
C	245 (95.70)	176 (93.62)	1	-	
T	11 (4.30)	12 (6.38)	1.519	0.655-3.520	0.327

OR = odd's ratio; CI = confidence interval.

Table 6. Phenotypes resulting from SNPs in the polymorphism site rs35343655 of the *Fn* gene.

Subjects	Cases (N = 128)	Control (N = 94)	OR	95% CI	P value
Genotype (%)					
A/A	42 (32.81)	20 (21.28)	1	-	
A/G	55 (42.97)	47 (50.00)	1.795	0.928-3.471	0.081
G/G	31 (24.21)	27 (28.72)	1.829	0.871-3.839	0.109
A/G+G/G	86 (67.18)	74 (78.72)	1.807	0.795-3.347	0.058
Allele frequency (%)					
A	139 (54.30)	87 (46.28)	1	-	
G	117 (45.70)	101 (53.72)	1.739	0.945 ~ 2.012	0.095

OR = odd's ratio; CI = confidence interval.

DISCUSSION

Currently, genes polymorphism analyses have seen increased usage in the study of the molecular mechanism of calcium oxalate stones, and has been applied to discover the relationship between the occurrence of calcium oxalate stones and polymorphisms in the γ-hydroxybenzyl thiocyanide glutamic acid, vitamin D receptor, and calcitonin receptor genes, among others (Bid et al., 2005; Gao et al., 2007; Metin et al., 2009). Patel and Lodish (1987) mapped the *Fn* gene to chromosome 2q34-36, and reported the presence of 50 exons. A majority of these exons corresponded to the repeat sequences of peptide chains. RFLP analysis, using the *Hin*dIII, *Hae*IIIb, *Taq*Ia, *Taq*Ib, *Msp*I, and *Hae*IIIa enzymes, helped confirm the presence of 6 genotypes. A number of studies have attempted to elucidate the relationship between the *Fn* gene and tumor susceptibility, metastasis, bone tissue mineralization, damage repair, liver and pulmonary fibrosis, and end-stage renal disease, among others. Tsujihata et al. (2000, 2001, 2006) reported that *Fn* significantly inhibited the growth and aggregation of calcium oxalate crystals *in vitro*; in addition, this gene was believed to impart the ability of forming a biofilm on the surface of calcium oxalate crystals, subsequently inhibiting the calcium oxalate crystal-biofilm combination using renal tubular epithelial cells (Tsujikawa et al., 2007; Tsujihata, 2008). Okada et al. (2010), on the other hand, reported that the inhibition of *Fn* in calcium oxalate crystal-forming animal models induced migration, phagocytosis, and digestion of monocytes and macrophages on calcium oxalate crystals, as a result of the interaction between Fn and CD44.

Three polymorphism sites in the *Fn* gene (SNPs rs6725958, rs10202709, and rs35343655) were mapped in this study. The genotype and gene frequency distributions of all three were consistent with the Hardy-Weinberg equilibrium in both the case and control groups, which indicated that the samples could be representative of the Uighur population residing in the Xinjiang area of China. The results revealed the absence of any imbalance between the sites. No significant difference was observed in the risk analysis between the genotype, allele, and occurrence of calcium oxalate stones (Tables 4, 5, and 6).

Onaran et al. (2009), in a study involving 143 patients with calcium oxalate nephrolithiasis and 154 healthy volunteers, discovered that polymorphisms in the *Hin*dIII restriction site of the *Fn* gene were significantly related to the development of calcium oxalate stones. However, a thorough comparison of the cases and controls revealed the absence of any significant differences the genotype and allele frequency in the three *Fn* sites (rs6725958, rs10202709, and rs35343655) in this study. This indicated that polymorphisms in the rs6725958, rs10202709, and rs35343655 sites of the *Fn* gene were not representative of the risk factors for the incidence of calcium oxalate stones in the Uighur population of the Xinjiang region of China. This study revealed significant

differences between susceptibility genes, based on the race of the study subjects, methods of analysis, and sample size; this indicates the need for further research in this field. Therefore, the results of this study could not explain the effect of *Fn* in the formation of calcium oxalate stones in patients belonging to the Uighur population of Xinjiang, China.

Gene polymorphisms occurring at the three SNP sites in the *Fn* gene were analyzed in this study; however, more relevant clinical data must be collected, the genotype-phenotype relationship must be further analyzed (statistically; with an expanded sample size and between different races), for further confirmation and elucidation of the results obtained in this study. The distribution of polymorphisms in the three sites in the *Fn* gene must also be carefully analyzed in order to determine the effect of polymorphisms on the development of calcium oxalate stones in urinary calculi patients.

Conflict of interest

The authors declare no conflict of interest.

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