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A polymorphism of matrix Gla protein gene is associated with kidney stone in the Chinese Han population

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ABSTRACT

Purpose: Matrix Gla protein (MGP) is a molecular determinant regulating the extracellular matrix calcification. To further confirm whether the MGP genetic polymorphism was universally associated with the risk of kidney stone, we investigated the association of genetic polymorphisms of MGP with kidney stone in the Chinese Han population.
Materials and methods: 728 subjects were recruited for the study. We firstly re-sequenced the human genomic *MGP* gene including the 1500 bp promoter, 5′-UTR, 4 exons and 3′-untranslated regions, identified single nucleotide polymorphisms (SNPs) in *MGP*, and performed an association analysis with kidney stones in 54 subjects of the Chinese Han population. A candidate tag SNP was genotyped in total subjects using an allele specific PCR, and further analyzed the association with kidney stone.
Results: We identified 18 polymorphisms including four tag SNPs. A tag SNPrs4236 was associated with kidney stones. The G allele carrier had a 1.373-fold reduced kidney stone risk compared with A allele carriers in SNPrs4236 (odds ratios (OR) = 1.373; 95%CI, 1.051–1.793; $p = 0.019$). However, we did not find an association between the polymorphism and clinical characteristics of kidney stones.
Conclusions: Our findings showed that SNPrs4236 of the *MGP* gene is associated with kidney stones in the Chinese Han population, and influences the genetic susceptibility to kidney stones. In the future, functional assays of the polymorphism should permit a better understanding of the role of MGP genetic variants and kidney stones.

1. Introduction

Kidney stone is a complex disease resulting from an interaction between environmental and genetic factors. About 2–5% of the population in Asia and 8–15% in Europe and North America develop renal stones in their lifetime (Pak, 1998). Wide geographical variations and racial differences exist in stone incidence and composition. Racial genetic polymorphisms may affect intricate interactions between promoting and inhibiting stone formation factors in renal tubules and are associated with the risk of kidney stone disease. We previously identified a variant of the human *MGP* (matrix Gla protein) gene associated with the individual sensitivity to kidney stone disease within the Japanese population (Gao et al., 2007). This association needs to be confirmed by further replication studies, particularly in other ethnic populations. The differences in risk allele frequencies and linkage disequilibrium (LD) structure across ethnicities can provide further insights to exact the association information and identify the true risk variant.

MGP is a molecular determinant regulating calcification of the extracellular matrix, and is expressed at high levels in the kidney, bone, lung and heart. A previous study showed that *MGP* expression was higher in calcified human atherosclerotic plaques and inhibited calcification (Canfield et al., 2002; Proudfoot et al., 1998). Luo et al. showed that homozygous *MGP*-deficient mice were observed to die within 8 weeks as a result of arterial calcification that led to blood vessel rupture (Luo et al., 1997). Kidney stone is a common ectopic calcification similar to vascular calcification, such as forming calcific plaques, increasing expression of calcification inhibitors and regulating actively calcification process. *MGP* gene expression was detected to maintain a high level in the renal tubular epithelial cells (Fraser and Price, 1988; Wang et al., 2000; Yasui et al., 1999), and was upregulated following an exposure to calcium oxalate monohydrate (COM) and oxalate (Gao et al., 2010). Lian et al. reported that a

Abbreviations: MGP, Matrix Gla protein; SNPs, Single nucleotide polymorphisms; htSNP, Haplotype tagging SNP; OR, Odds ratio; CI, Confidence interval; LD, Linkage disequilibrium; COM, Calcium oxalate monohydrate; LR-PCR, Long range PCR.

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fragment of MGP, gamma-carboxyglutamic acid was found in the calcium-containing kidney stone matrix (Lian et al., 1977). These observations show that MGP is not only an important biomarker of atherosclerotic calcification, but may also be associated with stone formation in the kidney.

Understanding the genetic basis of complex human diseases has been increasingly emphasized. The human *MGP* gene is located at 12p13.1–p12.3 and consists of 4 exons (Cancela et al., 1990). Domains of *MGP* corresponding to each exon were found in all known vitamin K-dependent vertebrate proteins: a transmembrane signal peptide, followed by a putative gamma-carboxylation recognition site and a Gla-containing domain that have a high affinity for calcium, phosphate ions and hydroxyapatite crystals (Price, 1989). To confirm whether the MGP genetic polymorphism was universally associated with the risk of kidney stone, we further investigated the genetic polymorphisms of MGP in the Chinese Han population in the present study.

2. Materials and methods

2.1. Study subjects

Our study group consisted of a total of 354 unrelated Chinese patients (mean age ± SD 54.3 ± 12.8 years) with kidney stones, who underwent treatment at Shenyang Medical College Hospital from 2008 to 2011. The clinical characteristics of all of the patients are listed in Table 1. Stone number was confirmed by X-ray before treatment, and was considered "multiple" when the stone number was more than two. The stone composition was analyzed by infrared spectroscopy. The primary age and stone frequency were investigated through medical records. Considering the complex etiology and different pathogenesis, we rigorously eliminated kidney stone cases with hyperparathyroidism, urinary tract infection and non-calcium salt stones with serial blood and urine biochemistry assays and stone composition analysis, respectively.

The control group consisted of 374 Chinese subjects (mean age ± SD 58.6 ± 11.5 years, 296 males and 78 females) without a history of kidney stones or a family history of stone disease. All of these control subjects were given a general medical inquiry and partially confirmed with X-ray, and routine blood and urine assays. All of the patients and controls were attributed to the Chinese Han race and the same geographical and environmental stratification. The protocol was previously approved by the Institutional Review Boards, and the appropriate informed consent was obtained from all patients and control subjects in this study. Genomic DNA from patients and controls was extracted from peripheral blood using a Blood Genome DNA Extraction Kit (Takara).

Table 1
Characteristics of 354 kidney stone patients.

Characteristics	No. pts (%)
Sex	
Male	289 (81.6)
Female	65 (18.4)
Primary age	
≤50 years	153 (43.2)
>50 years	201 (56.8)
Stone No.	
Multiple	147 (45.9)
Single	173 (54.1)
Stone frequency	
Primary	135 (38.1)
Recurrence	219 (61.8)
Stone component	
CaOx	163 (74.6)
CaP	55 (25.3)

2.2. Long range PCR amplification

Long range PCR spanning the *MGP* gene sequence was performed using the Platinum *Taq* DNA Polymerase High Fidelity Kit (Invitrogen, USA), according to the manufacturer's guidelines on genomic DNA samples from 26 cases and 28 controls. Briefly, 300 ng of genomic DNA was used as a template in 50 μl reactions. Specific amplification primers were designed as those of our previous study (Gao et al., 2007). The final concentrations of reagents included 2 mM $MgSO_4$, 0.2 mM dNTPs, and 0.2 μM of each primer, 2.5 units of Platinum *Taq* DNA Polymerase High Fidelity and 10-fold diluted High Fidelity PCR buffer. PCR amplification was performed with 1 cycle at 94 °C for 30 s, and 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 68 °C for 11 min. The products were resolved on 1.5% agarose gels and purified with EasyPure Quick Gel Extraction Kit (TransGen Biotech, Beijing).

2.3. Genotyping

LR-PCR products were re-sequenced. Re-sequencing PCR amplifications were performed in 26 cases and 28 controls. Walking primers were designed as those of our previous study (Gao et al., 2007). All sequencing PCR reactions were performed with the Applied Biosystems (ABI) BigDye Terminator Cycle sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's guidelines. Fifty nanograms of purified LR-PCR products were used in each cycle-sequencing PCR reaction. Cycle sequencing parameters were 25 cycles at 95 °C for 15 s, 55 °C for 15 s, and 60 °C for 4 min. The reaction products were ethanol precipitated, re-suspended in formamide-loading buffer, and electrophoresed on an ABI 3700 automated DNA sequencer. Sequences were aligned to compare with the published genomic sequences among all individuals, and SNPs were validated with the SeqScape program if the 2nd mixed bases were ≥45% of the highest peak. One candidate tag SNP was further genotyped in the additional 328 kidney stone cases and 346 controls using an allele special PCR assay. In brief, a forward primer, 5′-CAGGCTAGTCTTGAACTCC-3′ and two reverse primers, 5′-TTCCCTCA GTCTCATTTAGT-3′ and 5′-TTCCCTCAGTCTCATTTAGC-3′ were designed. PCR was performed in a total volume of 25 μl, containing 100 ng of genome DNA, 200 nM of forward primer, and 200 nM of each reverse primer. The amplification reaction was carried out with 1 cycle at 94 °C for 30 s, and 25 cycles at 94 °C for 30 s, 51 °C for 30 s and 72 °C for 30 s.

2.4. Statistical analysis

The chi-square test method was used to calculate the Hardy–Weinberg equilibrium for each of the polymorphisms. The Haploview software package (http://www.broad.mit.edu/mpg/haploview/about.php) was used to estimate the pair-wise linkage disequilibrium with coefficient D′ and r^2, and identify haplotype tagging SNPs (htSNP) using a confidence interval algorithm. The allele distribution of each SNP with a minor allele frequency greater than 1% was assessed by the chi-square test between the case and control samples. Odds ratios and 95% confidence intervals were calculated to determine the risk of kidney stone with a given allele. Statistical comparisons for genotype distributions were performed between the cases and controls with the chi-square test. Statistical analysis was performed using SPSS (version 10.0; SPSS Inc., Chicago, Illinois, USA). A p value of <0.05 was considered to be significant.

3. Results

3.1. Polymorphisms and linkage disequilibrium analysis in the human MGP gene

The human *MGP* gene was re-sequenced in 26 cases and 28 controls. A total of 18 polymorphism loci were genotyped (Fig. 1, Table 2). All