Investigation of the association of G-7A and T-138C single nucleotide polymorphisms on the promoter of MGP gene with renal stone

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ABSTRACT

Introduction: Renal stones, due to their heavy costs of diagnosis and treatment, have a considerable financial burden on health system. Family history and genetic susceptibility are important factors in stone formation. Matrix Gla-protein (MGP) is a gene that has a known role in inhibition of arterial calcification. It is thought that MGP may be involved in pathogenesis of calcium nephrolithiasis as an ectopic calcification.

Objectives: This study aims to demonstrate association of G-7A and T-138C single nucleotide polymorphisms (SNPs) of this gene with kidney calcium stone in Iranian population.

Patients and Methods: Seventy-nine patients with renal stone who underwent PCNL or open surgery were enrolled. Blood samples from each patient and his/her parents were taken and DNA extraction was done using salting out and phenol-chloroform extraction protocols. Genotypes for the MGP T-138C and G-7A polymorphism were determined by PCR-sequencing methods. Statistical analysis and transmission disequilibrium test were done by SPSS software.

Results: Total of 235 DNAs were extracted. The most frequent nucleotide is G (67%) in G-7A SNP and T (69%) in T-138C SNP. The CA haplotype was not seen in observed population and other haplotypes had same frequencies. No over transmission of alleles in two SNPs and no association of MGP Polymorphisms and nephrolithiasis were observed.

Conclusion: This study is the first family based investigation in Iranian population, which shows, no specific pattern of transmission and no effect of inherited SNPs on stone formation.

Keywords: Renal stone
Single nucleotide polymorphisms
Kidney

Implication for health policy/practice/research/medical education: This study is the first family based investigation which was done in patients with kidney calcium stone, demonstrate association of G-7A and T-138C single nucleotide polymorphisms (SNPs) of this gene in Iranian population. This study showed higher frequencies of G and T alleles in -7 and -138 SNPs of MGP gene, respectively. However, no specific pattern of transmission was seen and inherited SNPs have no effect on stone formation.

Material and Method

Patient selection
Renal stone forming patients underwent PCNL or open surgery between November 2008 and March 2010 at the Labafinejad hospital, Tehran, Iran, were selected. To be included in the study, patients should have at least one calcium oxalate stone and alive and accessible parents. Patients with history of other known familial disease, history of taking long-term medications or known obstruction in urinary system were excluded. Each patient and his/her parents were studied as a triose. All patients were informed about the study and satisfaction form was filled by them.

DNA extraction
Blood samples were taken at the Labafinejad hospital and transported to the laboratory where DNA extraction was performed using salting out and phenol-chloroform extraction protocols. Extracted DNA was quantified spectrophotometrically by NanoDrop (Thermo Fisher Scientific, Wilmington, USA).

PCR amplification
PCR was performed in a 35ml reaction volume containing 0.3M of each sense and antisense primer, as below:
- Sense primer: 5’-AAGCATACGATGGCCAAAACTTCTGCA-3’
- Antisense primer: 5’-GAACGATTCGAGAACCTCCTGCAACCC-3’

The amplification reaction was done using 0.2 mM of dNTP, 10X PCR buffer, 2.5 mM MgCl2, and 3U Taq DNA polymerase. The running conditions were predenaturat at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and synthesis at 72°C for 1 minute. Final extension was conducted at 72°C for 5 minutes.

Genotyping
Genotypes for the MGP T-138C and G-7A polymorphism were determined by PCR-sequencing methods.

Ethical issues
The research followed the tenets of the Declaration of Helsinki. Informed consents were obtained. All patients took part in this study voluntary. The research was approved by ethical committee of Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences. This study was extracted from residential thesis of Babak Ahadi (# 90-126).

Statistical analysis
The transmission pattern of alleles from parents to the child was compared by the Transmission disequilibrium test (TDT). Relative risk was estimated by the calculation of the odds ratio (OR) with 95% confidence interval. SPSS software was used for analyzing demographic data and R software for TDT estimation.

Results
Total of 235 DNAs were extracted from 79 patients’ and their parents’ blood samples. (Two patients were siblings). Fifty-two patients (65.82%) were male and 27 patients (34.18%) were female. There are two forms of nucleotides (A and G) in -7 SNP so 3 different genotypes are probable in this SNP (AA, AG and GG), also there is C or T in -128 SNP and the probable genotypes would be CC, CT and TT. The most frequent nucleotide is G in G-7A SNP and T in T-138C SNP (Table 1). Heterozygous genotypes (AG and CT) are the most frequent genotypes in both SNPs (Table 2). Table 3 demonstrates the frequencies of genotypes.

Table 1. Frequencies of observed SNPs

<table>
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<tr>
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</tr>
<tr>
<td>G</td>
<td>317</td>
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<td>A</td>
<td>153</td>
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<tr>
<td>G</td>
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<td>33</td>
</tr>
<tr>
<td>A</td>
<td>153</td>
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<td>T</td>
<td>323</td>
<td>69</td>
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<tr>
<td>C</td>
<td>147</td>
<td>31</td>
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Table 2. Frequencies of genotypes in observed SNPs

<table>
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<tr>
<th>SNP</th>
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<tr>
<td>-7</td>
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<tr>
<td>CT</td>
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<td>39</td>
</tr>
<tr>
<td>CC</td>
<td>26</td>
<td>11</td>
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</table>
the present study, we tried to investigate the association of 2 SNPs of this protein and stone formation in a family based study in Iranian population. However, our results indicated no significant association between the MGP polymorphism and nephrolithiasis. Also, analysis of data showed no over transmission of alleles in two SNPs (transmission disequilibrium test, -138 SNP TDT value ≈ 1.32, -7 SNP TDT value ≈ 0.37).

As an important factor in arterial calcification, polymorphisms of the MGP gene were studied in vascular calcification, and myocardial infarction. Herrmann et al. identified eight polymorphisms in the coding and 5′-flanking sequences of the MGP gene; among them A-7 polymorphism was associated with an increased risk of plaque calcification and MI (16). Although, in vitro analysis of MGP promoter activity revealed that the C-138 allele reduced promoter activity in cells but this polymorphisms has no significant impact on artery calcification (16,17).

Lu et al. identified 18 polymorphisms of MGP gene in Chinese Han population and they find an association between SNPsrs4236 and kidney stones in this population (13). There is a similar study in Japanese population which indicates the role of MGP and its polymorphisms in renal stone formation (14).

In Iran, Abiri et al. studied T-138C and A-7G polymorphisms in the MGP gene in patients with coronary artery disease (18). The findings of our study, which show a higher frequency for G allele in -7 SNP in investigated population is consistent with the findings of Abiri et al. study. However, the result in -138 SNP in our study show a higher frequency of T allele and in Abiri et al. investigation show a dominancy of C allele. This is notable that in Abiri et al. study the frequency of T allele in case group is insignificantly higher than C allele, which is now consistent with our findings and may indicate a role of T allele in calcification whether in arteries or in kidney.

Our result also showed no over transmission of alleles in two SNPs. This may be because of the sample size, therefore, higher sample size may be required to establish the role of transmission of alleles in kidney stone. On the other hand, to our knowledge, there is no other study to investigate the transmission of these alleles in Iranian population; more research on this topic needs to be undertaken before the association between SNPs and transmission of alleles is clearly understood.

**Conclusion**

This study is the first family based investigation which was done in patients with kidney calcium stone in Iranian population and showed higher frequencies of G and T alleles in -7 and -138 SNPs of MGP gene, respectively. However, no specific pattern of transmission was seen and inherited SNPs have no effect on stone formation. Future studies with a higher sample size and considering...
comparison between case and control group are recommended.

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Authors’ contribution
At conceived the study and contributed reagents and tools. BA, MT and MK performed the experiments. FZ, BN analyzed the data and drafted the final manuscript; all authors read, revised, and approved the final manuscript.

Conflicts of interest
There were no points of conflicts.

Ethical considerations
Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the author.

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References