Association of Vitamin D Receptor Gene Polymorphisms with Calcium Oxalate Calculus Disease*

WANG Shaogang (王少刚), LIU Jihong (刘继红), HU Shaqun (胡少群), YE Zhangqun (叶章群)
Department of Urology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030

Summary: To study the relationship between polymorphism of vitamin D receptor (VDR) allele with formation of calcium oxalate calculi and find the predisposing genes of calcium oxalate calculi, we screened out 150 patients who suffered from calcium oxalate calculi. 36 of them had idiopathic hypercalciuria according to analysis of calculus component and assay of urine calcium. The polymorphisms of VDR gene Taq1, Apa1 and Fok1 were detected using PCR-RFLP technique and the correlation were analyzed between the polymorphism and urinary calculus or between the polymorphism and hypercalciuria. The difference in each genotypic frequency of the allele of promoter Fok1 between calculus group and healthy group or between idiopathic hypercalciuria calculus group and healthy group was significant. The content of 24-h urine calcium of those who had genotype ff was obviously higher than that of those who have other genotypes in the same group. There was no significant difference in the polymorphism of gene Apa1 and Taq1 between each two groups. It is concluded that hypercalciuria and calcium oxalate calculus were related to the polymorphism of VDR gene's promoter Fok1 allele, but it had nothing to do with the polymorphism of gene Apa1 and Taq1. The genotype ff was a candidate heredity marker of calcium calculus disease.

Key words: gene polymorphism; vitamin D receptor; calcium oxalate calculus; hypercalciuria

Urinary calculus is a common disease that is closely related to the disorder of calcium metabolism and has the tendency of polygenic inheritance. But up to now its predisposing genes have yet to be identified. Vitamin D receptor (VDR) can regulate the metabolism of calcium and phosphorus in the body. Its allele polymorphisms are widely used to detect genetic characteristics of some diseases such as osteoporosis, rickets and secondary hyperparathyroidism [3-6]. In recent years, some researchers believed that there might be some relationship between VDR allele polymorphisms and urinary calculus or hypercalciuria. Therefore, we examined several familiar polymorphism sites of VDR gene with calcium oxalate calculi and hypercalciuria sufferers and analyzed the relation between each allele polymorphism and calcium oxalate calculus formation.

1 MATERIALS AND METHODS

1.1 Subjects
The subjects were divided into 3 groups. The patients in the first group had calcium oxalate calculi; the second had stones with hypercalciuria and the third group were healthy controls. 150 patients were selected who suffered from calcium oxalate calculi with normal urine calcium and were treated in our department from Nov. 2000 to Nov. 2001. There were 89 men and 61 women, with age ranging from 18-73 years, mean 43.6±16.4. All patients were eliminated from hypercalcemia, hyperuricemia, hyperlithuria and hyperparathyroidism by using blood and urine biochemical exam plus blood parathyrin exam. The possible hypercalciuria was excluded by 24-h urine calcium content (<0.1 mmol/kg). The calculus specimen was of calcium oxalate calculus as revealed by chemical analysis (The reagent kits were provided by the Department of Urology, Beijing University). The patients had no chronic urinary tract infection and renal function insufficiency. The second group had calcium oxalate calculi with 24-h urine calcium >0.1 mmol/kg including 22 men and 14 women. Ages was from 22—54 years, with an average of 36.0±11.7. This group had no abnormality in blood and urine except hypercalciuria. The control group included 80 healthy volunteers (58 men and 22 women). The age ranged from 20—79 years, with the average being 49±19.6. Subjects in this group had no family history of calculus and renal calcification. The ultrasonic examination and urinalysis revealed no abnormalities in urinary tract.

1.2 Methods
1.2.1 Reagents Peripheral blood DNA separation kit, TaqDNA polymerase and buffer, 4dNTPs, Taq1 restriction enzyme, Apa1 restriction enzyme, Fok1 restriction enzyme, DNA retrieving
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glass pearls kit and 100 bp’s product laddermarker were all products of MBI Fermentas company (Canada). The primer sequence was synthesized by the Shanghai Bioengineering Company on the basis of published VDR gene sequence by GeneBank, by using DNAmann program.

1.2.2 Methods DNA was extracted from peripheral blood by using DNA separation kit. A PCR system of gene polymorphisms of the promoter Fok1 contained DNA template, primers, 1× Taq polymerase buffer (1.5 mmol/L MgCl₂) and 0.5 μl Taq polymerase (final volume: 50 μl). The forward primers were 5’-ACTGACTCTGGCTCTGAC-3’ and the reverse primers were 5’-CACCTTGCTTCTTCTTCTCCC-3’. The PCR amplification cycling parameters included an initial denaturation at 94 °C for 5 min, then denaturation at 94 °C for 30 s, annealing at 57.5 °C for 45 s and extension at 72 °C for 45 s. The cycle was repeated 35 times, followed by an extension step at 72 °C for 7 min.

Detection of ApaI and TaqI polymorphisms used both the forward primers 5’-CAGAGCATG-CAGAGGGAC-3’ and the reverse primer 5’-AACAGCAACTCCTCAC-3’. The PCR system was the same as that for Fok1. The cycling included an initial denaturation at 94 °C for 7 min, then denaturation at 94 °C for 30 s, annealing at 54 °C for 45 s, and extension at 72 °C for 45 s. The cycle was performed 40 times with a final extension at 72 °C for 10 min. To increase purity, we cut gel after electrophoresis and retrieved DNA fragment by using DNA retrieving glass pearls. The specific restriction enzyme (5 μl) and its buffer was added into the products of PCR, which was stored overnight at 37 °C. Then DNA fragments were separated by gel electrophoresis.

2 RESULTS

2.1 Genotype of VDR

After gel electrophoresis, there was only one band (with a length of 745 bp) if there was no site for ApaI enzyme on allele of homozygote, and the genotype of the homozygote was designated as “AA”. There would be 3 bands (The length of them was 745 bp, 533 bp and 220 bp) if one site was present on allele of heterozygote, which was designated as “Aa”. Two bands would appear if two sites were on allele of homozygote (The length of them was 533 bp, 220 bp), which was designated as “aa” (fig. 1).

With TaqI fragment there were also three scenarios: TT homozygote (only one 745 bp band present), Tt heterozygote (three bands with length of 745 bp, 495 bp, 254 bp, respectively), and tt homozygote (two bands with the length 495 bp and 254 bp). The results of electrophoresis were shown in fig. 1.

Fok1 has three genotypes; FF, Ff and ff which are similar to the above-mentioned scenarios. The results of electrophoresis are showed in fig. 2.

2.2 Frequency of VDR Genotypes

The frequency of VDR genotypes of control group, group of calcium oxalate calculus with normal urine calcium and group of hypercalciuric calculus are listed in table 1. The distribution of VDR polymorphic genotypes of Apa1 and Taq1 had no significant difference between the last two groups and control group.

There was significant difference in Fok1 gene polymorphism of VDR between group of calcium oxalate calculus and control group and between group of hypercalciuric calculus and control group. But no significant difference was found between the two experimental groups. Table 2 showed that groups of calcium oxalate calculus and hypercalciuria calculus had high frequency of genotype ff.

2.3 The Relationship Between VDR Genotypes and Urine Calcium

The 24-h urine calcium of subjects with different genotypes is showed in table 3. There was a similar trend in the 3 groups, as shown by table 3. Urine calcium of genotype ff was significantly higher than that of genotype FF in the 3 groups (t-test, P < 0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>ApaI*</th>
<th>TaqI*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>Aa</td>
</tr>
<tr>
<td>Control</td>
<td>11 (13 %)</td>
<td>38 (48 %)</td>
</tr>
<tr>
<td>Calcium oxalate</td>
<td>32 (21 %)</td>
<td>69 (46 %)</td>
</tr>
<tr>
<td>Hypercalciuric</td>
<td>9 (25 %)</td>
<td>16 (44 %)</td>
</tr>
</tbody>
</table>

* No significant difference in distribution of genotypes was found among the three groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Types</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FF</td>
<td>FF</td>
</tr>
<tr>
<td>Control</td>
<td>17 (21 %)</td>
<td>44 (55 %)</td>
</tr>
<tr>
<td>Calcium oxalate calculus</td>
<td>27 (18 %)</td>
<td>64 (43 %)</td>
</tr>
</tbody>
</table>
| Hypercalciuric calculus | 5 (14 %)  | 13 (38 %) | 18 (50 %) | <0.05*

* No significant difference of distribution of genotypes was found between the two experimental groups