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Correlation study between the polymorphism of repetitive sequence in gene CAG of androgen receptor and the occurrence and progression of prostate cancer

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ABSTRACT

Objective: To explore the relation between the polymorphism of repetitive sequence in gene CAG of androgen receptor (AR) and the susceptibility and clinical stages as well as pathological grading of prostate cancer among Han population. **Method:** Sixty-eight cases with prostate cancer hospitalized in Urinary Surgery Department from Feb. 2010 to Feb. 2012 and 60 healthy cases were chosen as research subjects. Methods of PCR and direct sequencing were adopted to detect DNA sequence of AR gene and the length of repetitive sequence in CAG. **Results:** The lengths of repetitive sequence in CAG of patients with prostate cancer and healthy people were (22.3±4.6) and (23.0±4.9), respectively showing no statistical significance. Comparing length (repetitive sequence of CAG)>22, those with that < 22 suffer a remarkably higher risk of prostate cancer (P<0.05). The number of repetitive sequence in CAG of patients at clinical stage C-D was less than that of patients at stage B, and the number of repetitive sequence in CAG of patients with poorly differentiated prostate cancer was also less than that of patients with moderately and highly differentiated prostate cancer. But there was no statistical significance int the difference (P>0.05); the proportion of patients with length <22 at clinical stage C-D was much larger than that of patients at clinical stage B (P<0.05), and as the aggravation of pathological grading, the proportion of patients with the length <22 was also remarkably increased and there was significant difference between patients with highly differentiated prostate cancer and those with poorly differentiated prostate cancer (P<0.05). **Conclusions:** There is correlation between the occurrence and development of prostate cancer in Han population and the polymorphism of repetitive sequence in gene CAG of androgen receptor. The less the number of repetitive sequence in CAG is, the higher the risk of prostate cancer will be and the more severe the clinical stage and pathological grading will be.

1. Introduction

Prostate cancer (Pca) is a malignant tumor of urinary system with remarkably biological genetic characteristics and the rate of its occurrence is high in Europe and the United State[1] but it is low in Asia. So far, its exact pathogenesis and the reason of different prevalence among different races are not clear. Studies in recent years showed that the gene polymorphism of androgen receptor (AR), especially the polymorphism of repetitive sequence in CAG (the first exon), is closely related to the occurrence and development of prostate cancer[2-5]. But most of the studies focused on the population in Europe and the United State where the risk of prostate cancer is high. Study reports about the polymorphism of related susceptibility gene of prostate cancer in Han population in our country are few. To make a further study about the relation between the polymorphism of repetitive sequence in CAG of androgen receptor in Han population and the biological characteristics of prostate cancer, we made a comparison about the polymorphism of repetitive sequence in CAG among the patients at the same clinical stage and with different pathological grading and healthy people.

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2. Materials and methods

2.1. General materials

Sixty-eight patients who came to urinary surgery department of our hospital during the period from Feb. 2010 to Feb. 2012 were enrolled. They were all diagnosed as prostate cancer by biopsy or operation and pathological examination. The range of their ages was 54-86 years and the average age was (67.4±7.8) years according to Whitmore-Jewett, there are 42 cases at stage B, 18 cases at stage C and 8 cases at stage D; according to Gleason evaluation standards, there are 48 cases with highly differentiated Pca (2-4 scores), 11 cases with moderately differentiated Pca (5-7 scores) and 9 cases with poorly differentiated Pca (8-10 scores). Sixty healthy cases (without the history of hyperplasia of prostate gland and prostate cancer) who came to the examination center in our hospital during the same period were chosen as control group. The range of their ages was 53-87 and the average age was (67.9±8.9) years. All the subjects were Han population and agreed to participate in the study.

2.2. Methods

2.2.1. Extraction of DNA in peripheral blood

Peripheral venous blood 4 mL was collected from each subject with a empty stomach. 0.2% EDTA-Na2 was used for anti-freezing. Improved salting out method[6] was used to extract DNA (Rapid extraction of genomic DNA Kit, Beijing SaiBaiSheng Gene Technology Co., Ltd.) and DNA was stored in fridge at -70 ℃.

2.2.2. PCR amplification of repetitive sequence in CAG

Two pairs of primers were synthesized according to Irvine RA methods[7,8] and two rounds of PCR reaction were performed on them. The upstream primer F1 was 5′-GTGCGCGAAGTGATCCAGAA-3′ and R1 was 5′-TCTG-GGACGCAACCTCTCTC-3′; the downstream primer F2 was 5′-AGAGGCCGCGAGCGCAGCAC-CTC-3′ and R2 was 5′-GCTGTGAAGGTTGCTGTTCCTCAT-3′. PCR reaction system 50 μL contains genomic DNA 100ng. Amplification parameters: the reaction conditions of the first round were 60s denaturation at 94 ℃, 60s annealing at 55 ℃ and stretch 90s at 72 ℃ and there were 17 circulations in total; the reaction conditions of the first round were 60s denaturation at 94 ℃, 60s annealing at 66 ℃ and stretch 90s at 72 ℃ and there were 28 circulations in total.

2.2.3. Analysis of amplified products

PCR products were placed on agarose gel for electrophoresis. The existence of objective gene fragment (200-250 bp) was confirmed. The products were purified (OMEGA Bio-tek Company Kit). The DNA sequence of products and the length of repetitive sequence in CAG were measured by ABI377DNA sequencer (Shanghai Boya Ltd.).

2.3. Statistic analysis

Software SPSS15.0 was used for statistical processing, measurement data were symbolized by $\bar{x}\pm s$, t test, x^2 test as well as single factor analysis of variance were used. Difference were considered as significant at $P<0.05$.

3. Results

3.1. Relation between polymorphism of repetitive sequence in CAG of AR and the susceptibility of Pca

The length of repetitive sequence in CAG of 68 patients with Pca was 16-31 and the average length was (22.3±4.6); length of that of healthy cases was 18-31 and the average length was (23.0±4.9). There was no significant difference in the average length of repetitive sequence between CAG of patients and that of healthy people ($t=0.83$, $P>0.05$). Using 22 as the critical value[9], we divided them into short CAG group (the repetitive sequence of CAG <22) and long CAG group (the repetitive sequence≥22). There was significant difference between the two groups ($x^2=3.97$, $P<0.05$) (Table 1).

3.2. Relation between the polymorphism of repetitive sequence in CAG of AR and the clinical stage of Pca

The average length of repetitive sequence in CAG of patients with prostate cancer at stage B was (22.8±3.1) and the average length of repetitive sequence in CAG of patients with prostate cancer at stage C-D was (21.5±3.5). There was no statistical significance in the difference ($t=1.58$, $P>0.05$). There were 15 cases (35.7%) in short CAG group at stage B and 27 cases (64.3%) in long CAG group; there are 17 cases (65.4%) in short CAG group at stage C-D and 9 cases (34.6%) in long CAG group. There was remarkable difference in the comparison between patients at stage B and those at stage C-D ($x^2=4.55$, $P<0.05$) (Table 2).

3.3. Relation between the polymorphism of repetitive sequence in CAG of AR and the pathological grading of Pca

The lengths of repetitive sequence in CAG of patients with pooly, moderately and highly differentiated prostate cancer were (20.3±3.6), (21.3±3.8) and (22.9±4.3), respectively. The length of repetitive sequence in CAG of patients with poorly differentiated prostate cancer was shorter than that of those with moderately and highly differentiated prostate cancer

Table 1

Comparison between the polymorphism of repetitive sequence in CAG of AR of patients with Pca and healthy people.

Group	n	Length of repetitive sequence in CAG	Distribution of repetitive sequence in CAG	
			<22	≥22
Pca group	68	22.3±4.6	32(47.1%)*	36(52.9%)*
Healthy group	60	23.0±4.9	17(28.3%)	43(71.7%)

Comparison between Pca group and healthy group:*$P<0.05$.

while there was no statistical significance in the difference (t=1.14, 1.70, P>0.05). The distribution of repetitive sequence in CAG of AR in short CAG group expanded as the pathological grading of prostate cancer aggravated. There was significant difference in the comparison between patients with highly differentiated Pca and those with poorly differentiated Pca (x^2=6.13, P<0.05) (Table 3).

4. Discussion

Prostate cancer is one of the malignant tumors in males. Its pathogenesis is closely related to androgen and the function of androgen is adjusted by androgen receptor (AR). AR is the member of the superfamily of steroid receptor and it mainly plays its role in N gene. The most remarkable characteristic of AR is that it contains one CAG with triad nucleotide repetitive sequences. The number of triad nucleotide repetitive sequences in CAG is 9-36 in healthy people[10-11]. The American Blacks have smallest number of that, the Whites rank the second and Asian Americans have the largest number of that, which just conforms to epidemiological investigation which showed the risk of prostate cancer in different races[12-14]. This may show that the polymorphism of repetitive sequence in CAG of AR is relevant with the occurrence and development of prostate cancer.

Currently, disputes about the relation between the polymorphism of repetitive sequence in CAG of AR and prostate cancer exist in both domestic and foreign studies. Some scholars argued that the less the number of repetitive sequence in CAG was, the higher the risk of prostate cancer would be[15-17]. Studies conducted by Akinloye and his colleagues[18] showed that every time the CAG fragment was increased by one, the risk of prostate cancer would be decreased by 3%. But other scholars believed that the length of repetitive sequence in CAG was not the risk factor of prostate cancer[19,20] and was not significantly related to the clinical stages and pathological grading of prostate cancer[21-23]. The studies on the population of Brazil conducted by Silva Neto B and his colleagues[24,25] showed that compared with that of normal people, the length of repetitive sequence in CAG of patients with prostate cancer was not remarkably shortened.

In our study, we firstly made a comparison about the polymorphism of repetitive sequence in CAG of AR between the 68 cases with prostate cancer and 60 healthy cases and found there was no significant difference in the average length of repetitive sequence in CAG in the two groups. To further understand the relation between the polymorphism of repetitive sequence in CAG and the susceptibility of prostate cancer, we, according to the reference and regarding 22 as standard[9], divided them into short CAG group (the repetitive sequence of CAG <22) and long CAG group (the repetitive sequence≥22). The results showed that the risk of prostate cancer in short CAG group increased remarkably (P<0.05), which conforms to most of foreign reports[26-30]. Then we studied the relation between the polymorphism of repetitive sequence in CAG of AR and the clinical stages and pathological grading of prostate cancer. The results showed that the number of repetitive sequence in CAG of patients at clinical stage C-D is less than that of patients at stage B, and the number of repetitive sequence in CAG of patients with poorly differentiated prostate cancer is also less than that of patients with moderately and highly differentiated prostate cancer. But there is no statistical significance in the difference, which may be caused by the small amount of samples. Still regarding 22 as the critical value, through comparison, it was found that the proportion of patients at clinical stage C-D in short CAG group is remarkably larger than those at stage B. As the aggravation of pathological grading, the proportion of patients with the length <22 is remarkably increased and there is significant difference between patients with highly differentiated prostate cancer and those with poorly differentiated prostate cancer.

To sum up, our study holds the opinion that there is relation between the occurrence and development of prostate cancer in Han population and the polymorphism of repetitive sequence in CAG of AR. The less the number of repetitive sequence in CAG is, the higher the risk of prostate cancer will be and the more advance the clinical stage and pathological grading will be. However, since the samples are relatively small, there is no statistical difference in the average length of repetitive sequence in CAG among groups, which needs to be further studied with more samples.

Table 2
Relation between the polymorphism of repetitive sequence in CAG of AR and the clinical stage of Pca.

Staging of tumor	n	Length of repetitive sequence in CAG	Distribution of repetitive sequence in CAG	
			<22	≥22
Stage B	42	22.8±3.1	15(35.7%)	27(64.3%)
Stage C-D	26	21.5±3.5	17(65.4%)*	9(34.6%)*

Comparison between patients at stage C-D and those at stage B:*P<0.05.

Table 3
Relation between the polymorphism of repetitive sequence in CAG of AR and the pathological grading of Pca.

Pathological grading	n	Length of repetitive sequence in CAG	Distribution of repetitive sequence in CAG	
			<22	≥22
Poor differentiation	9	20.3±3.6	8(88.9%)	1(11.1%)
Moderate differentiation	11	21.3±3.8	6(54.5%)	5(45.5%)
High differentiation	48	22.9±4.3	18(37.5%)	30(62.5%)*

Compared with those poor differentiation:*P<0.05.

Conflict of interest statement

We declare that we have no conflict of interest.

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