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# Restriction fragment length polymorphism of pepsinogen C gene in patients with gastric carcinoma and in high risk population of gastric carcinoma

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## Abstract

**AIM:** To analyze the difference of distribution of the pepsinogen C (*PGC*) gene polymorphism among different groups and to study its application value in screening of the high risk population of gastric carcinoma and its value as an indicator for gene diagnosis.

**METHODS:** The study consisted of two parts: study on the restriction fragment length polymorphism (RFLP) of *PGC* gene in normal individuals and patients with gastric cancer, and study on the *PGC* gene polymorphism in members from a family at a high risk for stomach carcinoma and the follow-up survey. A total of 40 cases were analyzed including 11 healthy blood donors, 10 gastric carcinoma patients, and 19 members from a high risk family of stomach carcinoma. The Southern blot method was adopted in the study. The probe and restriction endonuclease used were *PGC* 301 and *EcoR* I, respectively. Gastroscopy and gastric mucosa biopsy were performed in the follow-up study.

**RESULTS:** There were three kinds of common *PGC EcoR* I allelic fragments (20 kb, 5.7 kb and 3.6 kb) and only one rare fragment (3.5 kb) in the normal subjects, and there was no difference in allelic fragments between the normal subjects and the patients. The incidence of the rare fragment and rare hybrid band type in the patients was higher than that in the normal subjects. The incidence of rare fragment and rare hybrid band type in the members from a high risk family was a little higher than that in normal subjects. After the 3-year follow-up by gastroscopy, one of the four members from the high risk family with *PGC EcoR* I rare fragment was found to suffer from early stomach cancer. The hybrid signal of *EcoR* I common allelic fragments in the gastric tumor tissue was weakened, even disappeared.

**CONCLUSION:** *PGC EcoR* I rare fragment and rare hybrid band type in the early diagnosis and screening of high-risk population of stomach carcinoma are probably of important application value. There is gastric normal differentiation gene (*PGC* gene) deletion in the stomach tumor tissue.

**Key words:** Stomach neoplasms; PGC; Restriction fragment length polymorphism



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## INTRODUCTION

In recent ten years, with the application of molecular biological techniques in clinical medicine, as a method of gene diagnosis, the applying value of restriction fragment length polymorphism (RFLP) analysis in the diagnosis of cancer as well as the screening of high risk population of cancer has called wide attention of scholars. In 1985, Krontiris *et al*[1] first reported that the polymorphism of ras gene could be used in predicting the risk of cancer occurrence. From then on, there have been many studies focusing on the polymorphism analysis of tumor genes in hopes of finding out the specific genetic susceptible marker of cancer[2-5]. However, in few studies the polymorphism was analysed using some normal tissue differentiation genes. In fact, compared with the common tumor gene detection method, the polymorphism analysis of some normal tissue differentiation genes is even more specific for the diagnosis of some kinds of cancer.

In 1988, Azuma *et al*[6] first reported the polymorphism of pepsinogen C (*PGC*) gene. In 1993, Azuma *et al*[7] studied the ulcer

**Table 1 The distribution of pepsinogen C 301 alleles**

| Allele | Size (kb) | Normal subjects *n* (%) | Patients *n* (%) |
|---|---|---|---|
| a1 | 20.0 | 22 (100) | 16 (80) |
| a2 | 5.7 | 22 (100) | 20 (100) |
| a3 | 3.6 | 20 (90) | 14 (70) |
| a4 | 3.5 | 2 (10) | 6 (30) |

**Table 2 The distribution of DNA band types**

| Hybrid band type | Normal subjects *n* (%) | Patients *n* (%) |
|---|---|---|
| Common type | | |
| a1/a2/a3 | 9 (82) | 4 (40) |
| Rare type | | |
| a1/a2/a3/a4 | 2 (8) | 3 (30) |
| a1/a2/a4 | 0 | 1 (10) |
| a2/a3/a4 | 0 | 1 (10) |
| a2/a3 | 0 | 1 (10) |

**Table 3 The distribution of pepsinogen C 301 alleles**

| Allele | Size (kb) | Family members *n* (%) |
|---|---|---|
| a1 | 20.0 | 38 (100) |
| a2 | 5.7 | 38 (100) |
| a3 | 3.6 | 36 (86.8) |
| a4 | 3.5 | 5 (13.2) |

**Table 4 The distribution of DNA band types**

| Hybrid band type | Family members *n* (%) |
|---|---|
| Common type | |
| a1/a2/a3 | 15 (78.94) |
| Rare type | |
| a1/a2/a3/a4 | 3 (15.79) |
| a1/a2/a4 | 1 (5.26) |

located in the body of the stomach using the *PGC* polymorphism, and found that the frequency of *PGC EcoR* I rare fragment of the patients was significantly higher than that of the normal population. They thought that the *PGC* polymorphism could be regarded as a genetic susceptible marker of ulcer in the body of the stomach. So far there has been no report on the *PGC* polymorphism in patients with gastric carcinoma and in the high risk population. It is unknown if there is any difference between the polymorphism distribution of *PGC*, a gastric normal differentiation gene, in normal subjects and in patients with gastric carcinoma, and if the detection of the difference could be regarded as a marker in the early diagnosis of gastric carcinoma and in the screening of high risk population.

## MATERIALS AND METHODS

### *Materials*

The study consisted of two parts. The first part was study on the *PGC* gene polymorphism in normal individuals and in patients with gastric cancer. The second part was study on the *PGC* gene polymorphism in members from a high risk family of gastric carcinoma and the follow-up survey. A total of 40 cases were analysed. Ten gastric tumor tissues were obtained surgically at the First Affiliated Hospital of China Medical University, and 11 normal blood samples were obtained from healthy donors without family histories of gastric cancer. Nineteen peripheral blood samples for the study were obtained in February 1990 from a high risk family of gastric carcinoma in Zhuanghe City of Liaoning Province which is a high risk area of gastric carcinoma. By that time, there had been four deaths due to gastric carcinoma in three generations in the family. Except the aged and the children, 11 cases were examined by means of gastroscopic biopsy. The results showed that one case suffered from atrophic gastritis and the other 10 suffered from superficial gastritis of different stages. No case of gastric cancer was found.

### *Methods*

The Southern blot method was adopted in the study. The gene probe used in the experiment was *PGC* 301 which was supplied by Dr. Azuma of Kyoto Prefectural University of Medicine, Japan. Gastric cancer genomic DNA was extracted from the surgical specimens using sodium dodecyl sulphate (SDS), EDTA, proteinase K, phenol and chloroform. After removing RNA with RNA enzyme, DNA was precipitated with alcohol of two times in volume, mixed in proper TE buffer solution, and kept for use at 4 ℃. The DNA of normal people and members from a high-risk family was obtained from peripheral blood white cells. The extracting method was the same as that used in previous work[8]. Genomic DNA was digested with the stated restriction endonuclease *EcoR* I and electrophoresed on a 0.8% agarose gel. After electrophoresis, DNA was denatured, neutralized and transferred to nitrocellulose membranes according to the method of Southern blot. The α-32p dCTP PGC probe (3000 Ci/mmol, Amersham's product) was labelled using a Random Primer Labelling Amersham's Kit to a specific activity of $2 \times 10^9$ dpm/μg. Then, the nitrocellulose membranes were hybridized in a water bath at 42 ℃, agitated overnight, and washed, followed by autoradiography at -80 ℃ for 3 to 5 d.

## RESULTS

*EcoR* I RFLP analysis of *PGC* gene was performed in a total of 11 normal subjects, 10 patients with gastric carcinoma and 19 members from a high-risk family. The results showed that there were three kinds of common allelic fragments (20 kb, 5.7 kb and 3.6 kb) and only one rare fragment (3.5 kb) in the normal subjects. There was no difference in allelic fragments between the normal subjects and the patients, but the hybrid signal of common fragments of the patients was weakened and with deletion. Two patients had no 20 kb fragment, one had no 3.6 kb fragment, and five had 3.5 kb fragment (Table 1). The hybrid DNA bands were classified into five types. The common type was 20 kb/5.7 kb/3.6 kb which appeared in nine normal subjects and four patients. There were four rare types. One was 20 kb/5.7 kb/3.6 kb/3.5 kb which appeared in two normal subjects and four patients, and the other three were 20 kb/5.7 kb/3.6 kb/3.5 kb; 5.7 kb/3.6 kb/3.5 kb; and 5.7 kb/3.6 kb which appeared only in the patients (Table 2).

There were three generations and 19 members in the high risk family of gastric carcinoma. The first generation had 2 members, the second had 12 and the third had 5. After examination of the *PGC* gene *EcoR* I RFLP, we found that the incidences of the three common allelic fragments, 20 kb, 5.7 kb and 3.6 kb, in the family members were 100%, 100% and 86.6%, respectively. The 3.5 kb rare fragment appeared in four family members (Table 3). There were three hybrid band types in the family, one common type (20 kb/5.7 kb/3.6 kb) and two rare types (20 kb/5.7 kb/3.6 kb/3.5 kb and 20 kb/5.7 kb/3.5 kb) (Table 4). We examined by means of gastroscopic biopsy and pathology and followed the four family members (all in the second generation) who had *PGC* gene *EcoR* I rare fragment. Two and a half years later, one of them, a 54-year-old male, was found to have early gastric carcinoma. This man underwent gastroscopic examination and was diagnosed as having atrophic gastritis in March 1990. He also underwent the examination of *PGC* gene *EcoR* I RFLP in the same period, and the results showed that the band type was 20 kb/5.7 kb/3.6 kb/3.5 kb. In October 1992, gastroscopic reexamination revealed that there was an irregular superficial erosion with unclear border on the back wall of the upper part of the lesser curvature of the stomach body. It was 3 cm × 2 cm in size with gray-yellow mucus on its surface and hyperemic edge. The lesion was diagnosed as early stomach carcinoma of type $II_c$ and confirmed by gastric mucosa pathology as lowly differentiated carcinoma and signet ring cell carcinoma.

## DISCUSSION

It was reported[9,10] that *PGC* was located on 6p21.1-Pter with 9 exons and *PGC* 301 was a 1224-bp cDNA clone containing exons

2-9 of the human *PGC* gene coding sequence. There was *EcoR* I RFLP between exons 7 and 8 in normal subjects because of DNA polymorphism. The frequencies of its polymorphic fragment were 82.5% (3.6 kb) and 17.5% (3.5 kb). In the present study, the analysis of *EcoR* I RFLP of normal subjects showed that there were 20 kb and 5.7 kb fragments in all normal subjects, but 3.6 kb and 3.5 kb fragments were distributed in polymorphism. The allelic fragment of 3.6 kb was 90% while that of 3.5 kb was 10%, which was a little different from 17.5% reported in previous studies. The reason may be the small sample size and different subjects. By increasing the sample size, its allelic fragment might be more accurately illustrated. No 20 kb fragment was found in 2 (20%) patients with gastric cancer, which might be due to the partial deletion of the *PGC* gene in gastric cancer. This is probably of important significance in the occurrence of gastric cancer. In addition, the frequency of 3.5 kb polymorphism fragment in gastric cancer patients was significantly higher than that in normal subjects, and the frequency of rare band type in patients was also higher than that in normal subjects. The atrophic gastritis in a member of high-risk family with 3.5 kb fragment finally developed into early gastric cancer. The results suggest that it is necessary to increase the sample number to investigate the significance of *PGC* rare fragment and rare hybrid band type for the early diagnosis of gastric cancer, and to further compare the changes and polymorphism of *EcoR* I fragment in gastric tumor tissue and other tissues of the patient. These are essential for illustrating the significance of changes in *PGC* gene structure in the occurrence of gastric cancer.

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