A COMPARISON OF MICROSATELLITE INSTABILITY IN EARLY ONSET GASTRIC CARCINOMAS FROM RELATIVELY LOW AND HIGH INCIDENCE EUROPEAN POPULATIONS

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We have investigated the genetic basis of gastric carcinomas occurring in patients aged under 40 years from a Portuguese population with a relatively high incidence of gastric cancer. We analysed a panel of 12 microsatellite loci in DNA extracted from gastric carcinomas arising in 16 patients aged 24–39 years from Braga, Portugal. Overall, microsatellite instability (MI) in at least 1 locus was detected in 44% (7 of 16) of carcinomas. A single patient demonstrated a mutator phenotype suggestive of the hereditary nonpolyposis colorectal cancer syndrome with instability in 82% of loci. This carcinoma showed loss of expression of the hMLH1 mismatch repair protein. In a previous study, we found no evidence of MI among 10 cases of early onset gastric carcinomas from an English population, which has a relatively low incidence of gastric cancer. Comparing the 2 series, we found that there was a significant difference ($p = 0.04$) in the prevalence of MI (at least 1 marker affected). This geographical difference in low-level MI may be related to a significantly higher prevalence of background chronic atrophic gastritis (8 of 16 vs. 0 of 8) and *Helicobacter pylori* infection (15 of 16 vs. 2 of 8) in Portuguese carcinomas compared with English cases. Genetic mechanisms underlying the hereditary non-polyposis colorectal cancer syndrome may play a role in a small number of early onset gastric carcinomas. The difference in prevalence of low-level MI between these relatively high and low incidence European populations requires further investigation. *Int. J. Cancer* 85:189–191, 2000.
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In general, Western populations have a relatively low incidence of gastric cancer but there are some Western countries, such as Portugal and Finland, where the incidence of gastric cancer is relatively high (Neugut *et al.,* 1996). The genetic mechanisms responsible for these geographical differences are unexplored. Early onset gastric carcinomas (*i.e.,* arising in patients aged under 40 years) are usually diffuse, infiltrating, poorly differentiated and may be associated with poor prognosis (Mecklin *et al.,* 1988). Patients with early onset gastric carcinomas are uncommon and may have a hereditary aetiology such as a germline E-cadherin mutation or hereditary non-polyposis colorectal cancer (HNPCC; Vasen *et al.,* 1990; Guilford *et al.,* 1998).

HNPCC families are characterised by the early onset and a high frequency of colorectal, gastric and endometrial carcinoma when compared with the normal population. Carcinoma DNA obtained from HNPCC patients shows widespread microsatellite instability (MI) when compared with "normal" constitutional DNA from the same person (Aaltonen *et al.,* 1993; Peltomaki *et al.,* 1993). This replication error (RER) positive phenotype arises in the majority of cases due to mutation in the DNA mismatch repair genes *hMSH2* or *hMLH1* (Peltomaki and Vasen, 1997). An RER-positive phenotype has been detected in 47% of gastric carcinomas obtained from HNPCC families in Finland (Aarnio *et al.,* 1997). A small proportion of "sporadic" colorectal, gastric and endometrial carcinomas also demonstrate an RER-positive phenotype (Peltomaki *et al.,* 1993).

We aimed to compare the frequency of MI in early onset gastric carcinomas from European patients residing in areas of high (Braga, Portugal) and low (Yorkshire, UK) gastric cancer incidence. The frequency of RER positivity in the Braga patients may indicate whether HNPCC could account for the increased gastric cancer incidence in the area, possibly due to a founder mutation effect as seen in Finland (Papadopoulos *et al.,* 1994). We have previously investigated 10 gastric carcinomas arising in patients under the age of 40 from Yorkshire and found no evidence of MI (Hayden *et al.,* 1997b). We have used identical methods to investigate 16 patients aged under 40 years with gastric carcinoma from Braga, a region of Portugal with a relatively high incidence of gastric cancer, to determine whether there is a variation in the prevalence of RER positivity which may explain the geographical difference in incidence.

MATERIAL AND METHODS

Details of the patients

Paraffin-embedded tissue from 16 young gastric carcinoma patients was obtained from the Hospital de Sao Marco, Braga, Portugal. There were 10 male and 6 female patients aged 24–39 years at the time of diagnosis (median of 34 years). Carcinomas were located in the middle (n = 5) and distal third (n = 11) of the stomach (Table I). The cases were examined by an experienced gastrointestinal pathologist, as in Hayden *et al.* (1997b). The degree of differentiation was assessed (Watanabe *et al.,* 1990) and the histological appearances were categorised according to the classifications of Lauren (1965), Ming (1977), and Goseki *et al.* (1992). Tumour stage was assessed in accordance with the TNM classification system. The histological features of the "background" gastric mucosa and presence of *Helicobacter pylori* (modified Giemsa stain) were also recorded (Table I).

DNA extraction

Separate areas containing normal gastric mucosa or smooth muscle and corresponding gastric carcinoma (comprising at least 50% neoplastic cells) were outlined on a representative haematoxylin and eosin-stained section. Selected areas of tissue were microdissected from consecutive formalin-fixed sections and DNA was extracted using a standard phenol/chloroform method (Hayden *et al.,* 1997b).

Fluorescent PCR and detection of MI

Fluorescent PCR was performed using a panel of 12 microsatellite markers as previously described, for 30–40 cycles of amplification under optimised reaction conditions (Hayden *et al.,* 1997b).

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TABLE I – CHARACTERISTICS OF PORTUGUESE EARLY ONSET GASTRIC CARCINOMAS

Case number	Sex, age (years)	FH[1]	Location	Stage[2]	Lauren	Ming	Goseki	Grade[3]	Mucosa[4]	HP[5]	MI (%)[6]
1	M, 24	No	Antrum	I*	Intestinal	Infiltrating	IV	PD	CG, IM	No	0/8 (0)
2	M, 26	No	Body	I	Diffuse	Infiltrating	IV	PD	CAG, IM	Yes	1/5 (20)
3	M, 28	No	Antrum	III	Diffuse	Infiltrating	IV	PD	CG	Yes	0/11 (0)
4	M, 29	No	Antrum	III	Diffuse	Infiltrating	IV	PD	CG	Yes	1/6 (17)
5	M, 30	No	Body	IV	Diffuse	Infiltrating	IV	PD	CAG	Yes	1/6 (14)
6	F, 30	No	Antrum	I*	Diffuse	Infiltrating	IV	PD	CAG, IM	Yes	0/6 (0)
7	F, 31	No	Antrum	II	Diffuse	Infiltrating	IV	PD	CG, IM	Yes	1/10 (10)
8	M, 34	No	Antrum	I*	Diffuse	Infiltrating	IV	PD	CAG	Yes	1/4 (25)
9	M, 34	No	Body	III	Diffuse	Infiltrating	IV	PD	CAG	Yes	0/6 (0)
10	M, 35	No	Antrum	I*	Diffuse	Infiltrating	IV	PD	CG	Yes	0/5 (0)
11	F, 37	No	Antrum	III	Diffuse	Infiltrating	IV	PD	CG	Yes	0/7 (0)
12	F, 37	No	Body	III	Diffuse	Infiltrating	IV	PD	CAG, IM	Yes	0/8 (0)
13	F, 38	Yes	Antrum	I	Diffuse	Expansile	III	PD	CG, IM	Yes	9/11 (82)
14	M, 38	No	Body	III	Mixed	Infiltrating	IV	PD	CAG, IM	Yes	1/8 (13)
15	M, 39	No	Antrum	II	Intestinal	Infiltrating	I	MD	CAG, IM	Yes	0/10 (0)
16	M, 39	No	Antrum	II	Diffuse	Infiltrating	IV	PD	CG	Yes	0/6 (0)

[1]FH: family history of HNPCC-associated neoplasms.–[2]UICC classification (*carcinoma confined to the mucosa or submucosa).–[3]WHO classification: MD, moderately differentiated; PD, poorly differentiated.–[4]Background gastric mucosa. CG, chronic gastritis; CAG, chronic gastritis with atrophy; IM, multifocal intestinal metaplasia.–[5]*H pylori* identified.–[6]MI (%): number of MI-positive loci/number of markers yielding results (%).

The microsatellites used were: D2S123, D3S966, D3S1076, D5S82, D5S346 (DP1), D10S197, D11S904, D13S175, BAT25, NM23, p53 and DCC (Aaltonen *et al.,* 1993; Cawkwell *et al.,* 1994; Jones *et al.,* 1992; Liu *et al.,* 1995; Spirio *et al.,* 1993). All consisted of dinucleotide repeat motifs except BAT25 (mononucleotide) and p53 (pentanucleotide). PCR products were electrophoresed on a 6% polyacrylamide gel using an automated DNA sequencer model 373A (Applied Biosystems, Foster City, CA) and MI visualised using Genescan Analysis software (Applied Biosystems). MI was recorded when 1 or more additional peaks occurred in the carcinoma DNA sample which were absent from normal DNA from the same patient (Cawkwell *et al.,* 1995). Results were obtained for a minimum of 4 markers per sample.

Immunohistochemical staining for hMSH2 and hMLH1

Immunohistochemical staining for mismatch repair proteins was performed using monoclonal antibodies against hMSH2 (NA27, Calbiochem, Nottingham, UK) and hMLH1 (13291A, Phar-Mingen, San Diego, CA) as previously described (Cawkwell *et al.,* 1999). Immunohistochemical results were scored blind from the MI results for each case. Loss of expression was scored whenever staining was absent from tumour cells but present in adjacent non-neoplastic tissue.

Statistical analysis

All *p* values were calculated using Chi square with Yate's correction.

RESULTS

Each case was analysed with 12 microsatellite markers. Results were obtained for a minimum of 4, and an average of 7, loci per case. The overall frequency of MI in at least 1 locus was 44% (7 of 16) of carcinomas (Table I). Six cases (38%) showed low-level MI (only 1 marker affected). A single patient (6%) demonstrated a mutator phenotype suggestive of the HNPCC syndrome with instability in 82% of loci. This carcinoma exhibited normal hMSH2 expression but loss of expression of the hMLH1 protein when compared with adjacent normal mucosa, which stained positively with both antibodies.

The MI results were compared with our previous study of early onset gastric carcinomas from Yorkshire (Hayden *et al.,* 1997*b*). In this study, results were obtained for a minimum of 5, and an average of 9, loci per case. There was a significant difference in the prevalence of MI (at least 1 marker affected) between the patients from Braga and Yorkshire (6 of 16 *vs.* 0 of 10, *p* = 0.04). The assessment of background mucosa revealed a significantly higher incidence of *H. pylori* infection (15 of 16 *vs.* 2 of 8; *p* = 0.002) and chronic atrophic gastritis (8 of 16 *vs.* 0 of 8; *p* = 0.04) in the Braga cases. Of the 6 Braga cases which exhibited low-level MI, 4 had chronic atrophic gastritis and *H. pylori* infection. There were no significant differences in the prevalence of the remaining clinico-pathological features between the 2 groups.

DISCUSSION

The diagnosis of gastric carcinoma in patients under the age of 40 is a rarity in most European populations and is uncommon even in countries or regions with a relatively high incidence. Thus, of necessity, this study is based on a small number of cases.

Using a fluorescent PCR technique, we have found that 44% of early onset gastric carcinomas arising in patients from the Braga region of Portugal showed evidence of MI. An RER-positive phenotype, with associated defective hMLH1 expression, was detected in 1 female patient aged 38 years who had a 65 year-old first degree relative affected by gastric carcinoma. This patient may have a germline HNPCC mutation or, alternatively, the mismatch repair system may have been inactivated in the tumour by hypermethylation of the hMLH1 promoter (Leung *et al.,* 1999). None of the remaining patients had a family history of an HNPCC-associated carcinoma. Our results suggest that HNPCC may play a role in a small number of young patients with gastric carcinoma from high incidence European populations.

There have been contrasting reports concerning the prevalence of the RER-positive phenotype in gastric carcinomas from older patients from different populations. Studies of high incidence populations have reported RER positivity in 22–33% of cases (Chung *et al.,* 1996; Semba *et al.,* 1996). In contrast, series from low incidence countries have revealed a prevalence of 4–9% (Keller *et al.,* 1995; Hayden *et al.,* 1997*a*). Among young patients from both high and low incidence populations, we have found a paucity of RER-positive cases. This is less surprising since we and others (Keller *et al.,* 1995; Chung *et al.,* 1996; Hayden *et al.,* 1997*a*) have shown that RER-positive gastric carcinomas tend to be of the intestinal subtype, rather than diffuse. Therefore, a low prevalence of RER-positive cases may be expected in series comprising predominantly diffuse-type gastric carcinomas.

Although the number of cases in both series is low, we found a significant difference in the prevalence of MI, particularly at a low

level, between early onset English and Portuguese gastric carcinomas. This has not previously been reported and may warrant further investigation. Variation in the prevalence of low-level MI could be attributable to the significant difference in the level of background chronic atrophic gastritis which was observed. MI has been found in chronic pancreatitis (Brentnall *et al.,* 1995), ulcerative colitis (Brentnall *et al.,* 1996) and in intestinal metaplasia adjacent to gastric carcinomas (Semba *et al.,* 1996). It has been suggested that continuous cell regeneration in chronic inflammatory conditions may lead to saturation of the mismatch repair system, leading to uncorrected errors detected as MI (Brentnall *et al.,* 1996). It is known that *H. pylori* infection causes chronic atrophic gastritis (Correa and Shiao, 1994), inflicts oxidative DNA damage on gastric mucosa (Baik *et al.,* 1996) and has been linked with the development of gastric carcinoma (Forman *et al.,* 1993). Infection with this organism occurs at a high frequency among young patients from high incidence populations (Forman *et al.,* 1993) and this was found to be the case in the Portuguese series presented here. Chronic inflammation caused by *H. pylori* could result in the saturation of DNA repair mechanisms and conceivably lead to the development of low-level MI. The possibility that other environmental agents could give rise to this genetic abnormality requires further investigation.

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