

# Assessment of Microsatellite Alterations in Young Patients with Gastric Adenocarcinoma

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The authors are grateful to the Special Trustees of The Leeds General Infirmary for funding this study.

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Received August 14, 1996; revision received October 21, 1996; accepted October 21, 1996.

**BACKGROUND.** Genetic factors are probably important in the development of gastric carcinoma in young patients (younger than 40 years). The authors investigated early onset primary gastric adenocarcinomas for the presence of microsatellite instability, which is a phenotypic marker for the hereditary nonpolyposis colon carcinoma syndrome.

**METHODS.** DNA was extracted from archival microdissected carcinoma and corresponding normal tissue from 10 British gastric carcinoma patients age 19 to 39 years at the time of diagnosis. A panel of 12 microsatellite loci were amplified by fluorescent polymerase chain reaction and analyzed using an automated DNA sequencer.

**RESULTS.** There was no evidence of microsatellite instability. In contrast, allelic imbalance was recorded at D3S966, D3S1076, D10S197, D11S904, P53, NM23, and DCC microsatellite loci.

**CONCLUSIONS.** The authors reported ten cases of early onset gastric carcinoma that demonstrated allelic imbalance but no evidence of instability at microsatellite loci. It is unlikely that defective DNA mismatch repair is important in this group of young patients. *Cancer* 1997; 79:684-7. © 1997 American Cancer Society.

**KEYWORDS:** microsatellite instability, gastric adenocarcinoma, polymerase chain reaction, DNA mismatch repair.

**G**astric adenocarcinoma usually affects patients older than 50 years and rarely affects those younger than 40 years. Tumors occurring in young patients (younger than 40 years) account for < 5% of gastric carcinomas.<sup>1-5</sup> Genetic factors probably play a role in the development of this form of gastric carcinoma<sup>3</sup> but a mechanism has not yet been determined.

Recently, it has been shown that inherited defects in DNA mismatch repair genes predispose individuals to the hereditary nonpolyposis colorectal carcinoma syndrome (HNPCC).<sup>6-9</sup> Colorectal, gastric, and endometrial tumors from HNPCC kindreds exhibit replication errors that are manifested as microsatellite instability (MI).<sup>10</sup> Microsatellites are short nucleotide tandem repeat sequences that exhibit length polymorphisms.<sup>11</sup> MI may be detected as an increase or decrease in the length of a microsatellite sequence in tumor DNA when compared with normal DNA from the same person.<sup>10</sup>

DNA extracted from archival paraffin embedded tissue from a series of gastric carcinoma patients younger than 40 years was examined to determine the frequency of MI. Polymerase chain reaction (PCR) amplification of microsatellites was performed using fluorescent-labeled primers and the products analyzed using an automated DNA sequencer.<sup>12</sup>

**TABLE 1**  
**Clinicopathologic Characteristics of Early Onset Gastric Carcinoma Cases**

Case no.	Age (yrs)	Gender	Tumor site	Family history <sup>a</sup>	Stage <sup>b</sup>	Lauren	Ming	Goseki	Grade <sup>c</sup>	Background mucosa
1	19	M	Antrum	NA	IV	Diffuse	Inf	IV	Poor	N
2	21	F	Antrum	No	IV	Diffuse	Inf	IV	Poor/sig	NO
3	23	F	Body	No	I	Intestinal	Inf	IV	Poor	Hp, CG
4	25	F	Body	No	IV	Mixed	Inf	III	Poor/sig	N
5	27	M	Body	NA	III	Diffuse	Inf	IV	Poor	CG
6	28	M	Cardia	NA	IV	Diffuse	Inf	IV	Poor	NO
7	33	M	Antrum	No	I	Intestinal	Inf	I	Well	Hp, CG
8	35	M	Pylorus	No	III	Intestinal	Inf	IV	Poor	N
9	37	M	Body	No	III	Diffuse	Inf	III	Poor	CG, IM
10	39	M	GJ	No	IV	Mixed	Inf	IV	Poor	IM

M: male; F: female; GJ: gastroesophageal junction; NA: not available; No: no family history of gastric carcinoma or other cancer; Inf: infiltrating carcinoma; Sig: signet ring cells; N: normal; NO: not obtained; Hp: *Helicobacter pylori* identified by hematoxylin and eosin stain; CG: chronic gastritis; IM: intestinal metaplasia.

<sup>a</sup> A family history was obtained whenever possible.

<sup>b</sup> Tumors were staged as I-IV according to the International Union Against Cancer criteria.

<sup>c</sup> World Health Organization grading of well, moderate, or poor differentiation.

## MATERIALS AND METHODS

### Patient Characteristics

Ten white British patients younger than 40 years with gastric adenocarcinoma were identified from the Yorkshire Cancer Registry at Cookridge Hospital, Leeds, United Kingdom and The Department of Histopathology at The General Infirmary at Leeds. Clinicopathologic details are shown in Table 1. Gastric carcinomas were described with respect to the classifications of Lauren,<sup>13</sup> Ming,<sup>14</sup> and Goseki et al.<sup>15</sup> and graded according to the World Health Organization criteria.<sup>16</sup> Carcinomas were staged according to the International Union against Cancer (UICC) TNM classification system.<sup>17</sup> The histologic appearance of the 'background' mucosa was recorded whenever possible.

### DNA Extraction

Separate areas containing normal gastric mucosa or smooth muscle and corresponding gastric carcinoma (comprised of at least 50% neoplastic cells) from each case were outlined by a gastrointestinal histopathologist and microdissected into separate tubes containing 0.2 mg/mL Proteinase K (GIBCO BRL, Life Technologies Inc., Gaithersburg, MD). DNA was extracted using standard methods<sup>18</sup> and suspended in molecular biology grade water.

### Fluorescent PCR and Microsatellite Analysis

The authors synthesized fluorescent-labeled microsatellite primers that amplified D2S123, D3S966, D3S1076, D5S82 and DP1 (both linked to the adenomatous polyposis coli gene), D10S197, D11S904, D13S175, BAT25, NM23 (within the nm23 gene), P53

(within the p53 gene), and DCC (within the DCC gene).<sup>12,19-22</sup> With the exception of DCC, P53, and BAT25, all the loci were dinucleotide (CA)<sub>n</sub> repeats. DCC, BAT25, and P53 were (TA)<sub>n</sub>, (A)<sub>n</sub>, and (AAAAAT)<sub>n</sub> repeat motifs, respectively. PCR was performed for 35 cycles under reaction conditions described elsewhere.<sup>12,22</sup> Amplification products were electrophoresed on a 6% polyacrylamide gel using an automated DNA sequencer and visualized using Genescan Analysis software (Applied Biosystems, Foster City, CA).

MI was recorded when novel peaks occurred in the carcinoma DNA when compared with normal constitutional DNA from the same patient.<sup>12</sup> Allelic imbalance was determined as outlined previously.<sup>22</sup> Experiments demonstrating MI or allelic imbalance were confirmed by a repeat PCR.

## RESULTS

Table 2 summarizes the microsatellite alterations recorded in ten early onset gastric adenocarcinomas. Based on the assessment of at least five loci per case, there was no evidence of MI. In contrast, allelic imbalance was observed at D3S966 (1 of 3 informative cases), D3S1076 (1 of 4), D10S197 (1 of 4), D11S904 (1 of 6), P53 (2 of 7), NM23 (3 of 7), and DCC (2 of 4) microsatellite loci.

## DISCUSSION

Using fluorescent PCR and Genescan analysis, the authors analyzed 12 microsatellite loci in 10 early onset gastric carcinomas and found no evidence of MI. Several cases demonstrated allelic imbalance at microsatellite sequences, including those associated with the

**TABLE 2**  
**Summary of Microsatellite Alterations in Early Onset Gastric Carcinomas**

Case no.	D2S123	D3S966	D3S1076	D5S82	DPI	D10S197	D11S904	D13S175	BAT25	NM23	P53	DCC
1	HET	HET	U	HET	HOM	HOM	HET	HET	HOM	HOM	HET	HOM
2	U	U	U	HOM	U	HOM	U	U	HOM	HET	HOM	HOM
3	HET	U	HET	HET	HET	HET	HET	HET	HOM	HOM	HET	AI
4	U	U	U	HET	U	U	U	HET	HOM	AI	AI	U
5	HOM	HET	U	HET	HOM	HOM	HET	HET	HOM	HET	HOM	HET
6	HOM	HOM	AI	U	HET	AI	HOM	HET	HOM	AI	U	U
7	HOM	U	HET	HOM	U	HOM	U	HET	HOM	HOM	HET	U
8	HET	U	HET	HET	HET	HET	HET	HET	HOM	HET	HET	HET
9	HET	U	HOM	HET	U	HET	HET	HOM	HOM	HET	HET	AI
10	HOM	AI	U	HET	HOM	HOM	AI	AI	HOM	AI	AI	HOM

HET: heterozygous result; U: uninterpretable result; HOM: homozygous result; AI: allelic imbalance.

DCC, P53, and nm23 tumor suppressor genes. This may relate to the loss of heterozygosity observed at these loci that has been reported in sporadic gastric carcinomas<sup>23-25</sup> or reflect the spontaneous genetic deletions that occur during carcinogenesis.

Given the histopathologic features of the current series (Table 1), the absence of detectable MI was unexpected. In Japan, MI has been reported at 2 or more loci in 36% of advanced gastric carcinomas versus 17% of early stage tumors and 29% of intestinal-type carcinomas versus 39% of diffuse-type lesions.<sup>26</sup> Poorly differentiated gastric carcinomas are reported to have levels of MI as high as 64%.<sup>27</sup> However, recent Western series have demonstrated low levels of MI in gastric carcinoma. Keller et al found MI (at 2 or more loci) in only 2 of 46 cases (4%)<sup>28</sup> and Gleeson et al. identified a high frequency of MI (62% of loci were positive) in only 1 of 38 advanced stage carcinomas arising in the proximal third of the stomach (3%).<sup>29</sup> Further studies are required to determine whether the difference in the incidence of MI between Eastern and Western populations is due to methodologic or etiologic factors.

A possible reason for undetectable MI is that insufficient microsatellite loci were analyzed. Most studies of MI in gastric carcinoma examine < ten microsatellite markers per case.<sup>26,27,30-32</sup> Peltomäki et al have suggested that the absence of MI in three microsatellite loci is required to exclude the replication error positive phenotype.<sup>33</sup> One would expect to observe MI in at least 29% of loci to confirm an HNPCC-associated phenotype.<sup>10</sup> Therefore, the genetic basis of these early onset gastric carcinomas is unlikely to be related to HNPCC. The authors emphasize that the current series represents a small sample of this unusual group of

patients and therefore a much larger study is required to confirm these findings.

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