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Microsatellite Instability and Loss of Heterozygosity in Gastric Carcinoma in Comparison to Family History

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We compared 29 gastric carcinomas from patients with a variably strong family history for gastric cancer (group 1) with 36 gastric carcinomas from patients without a family history of this disease (group 2) for microsatellite instability (MSI) and loss of heterozygosity (LOH) with 12 microsatellite markers. Both study groups had similar proportions of histological types and tumor locations. Widespread MSI (alterations at ≥6 loci) was seen in 5 of 29 (17%) of the tumors belonging to group 1 and in 4 of 36 (11%) group 2 tumors. MSI at a low level (alterations at 1 to 3 loci) was observed in 12 of 29 (41%) of tumors in group 1 and in 10 of 36 (28%) of tumors in group 2, differences that were not statistically significant. A significant difference with respect to low level MSI was observed between the two groups when considering the overall mutation rate of microsatellites. Seventeen of 281 (6%) analyzed microsatellite loci showed alterations in group 1 and 11 of 381 (2.9%) in group 2 ($P = 0.046$). Comparison of both types of MSI to the clinicopathological parameters in both groups revealed a significant association of low level MSI with advanced tumor stages ($P = 0.046$) in the group 2, whereas no such association was observed in group 1. In respect to LOH, a significant difference between the two groups was observed at chromosome 17p12, as 13 of 22 (59%) informative cases of group 1 showed LOH in comparison with 7 of 26 (27%) ($P = 0.024$) in group 2. No correlation of LOH at chromosome 17p12 to the pathological or clinical data was observed either in the two groups or in the study as a whole. Our data show that gastric carcinomas of patients with a positive family history of gastric cancer in group 1 are characterized by a higher mutation rate in respect to low level MSI, particularly at dinucleotide repeats, and by a higher frequency of LOH at chromosome 17p12, indicating that different genetic pathways are involved in the pathogenesis of gastric carcinomas arising in patients with and without a familial background of this disease. ***(Am J Pathol 1998, 152:1281–1289)***

Although an overall decrease in the incidence of gastric cancer has been observed recently, it is still one of the most common malignancies worldwide.[1] The incidence of gastric carcinoma shows a great geographical variation with the highest frequencies being observed in Japan, China, and parts of South America.[2] The etiology of the disease and the mechanisms involved in its carcinogenesis are still poorly understood, but dietary habits and life style as well as bacterial infections have been suggested to be important in the tumorigenic process.[2] Epidemiological studies, mainly performed in Italy, have shown a significant association between gastric cancer risk and a positive family history of the disease in first degree relatives,[3–6] suggesting that besides the influence of common environmental factors, genetic factors may be involved in the pathogenesis of the tumor in some patients. Gastric carcinomas have been described to occur significantly more frequently in the hereditary nonpolyposis colorectal cancer syndrome, which is primarily characterized by the development of colorectal cancers.[7] Defects in DNA mismatch repair and germ-line mutations in four DNA mismatch repair genes have been identified to be the underlying genetic error in a majority of the patients with this syndrome.[8,9] The defects in DNA mismatch repair are reflected by a high degree of microsatellite instability (MSI) in the tumors of these patients.[10] MSI has also been demonstrated, although at a lower frequency, in sporadic tumors of various organs including gastric carcinomas.[11–24] Recently, a positive association of MSI with a family history of gastric carcinoma has been reported in an Italian study.[25]

In two previous studies, we also observed MSI more frequently in gastric cancer patients having a positive

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family history of the disease, counting one in eight loci positive for MSI.[19,26] In order to study this phenomenon in more detail and to differentiate for the type of instability with regard to the number of loci in relation to the number of loci tested, we extended the analysis to include 12 microsatellite markers, including various types of microsatellite repeats.

Microsatellites give information not only about MSI but, because of their highly polymorphic nature, also allow the detection of allelic deletions in tumors. Frequently deleted chromosomal regions suggest that these are sites of tumor suppressor genes involved in the carcinogenic process. To further characterize the genetic alterations occurring in the tumors of patients with and without a positive family history of the disease, we also evaluated the tumors for loss of heterozygosity (LOH). Because gastric carcinomas are not an homogenous tumor group and different patterns of genetic alterations have been implicated for the development of diffuse and intestinal type gastric carcinomas as well as for the development of tumors located in the proximal and distal stomach,[16,27,28] we compared a group with a family history of gastric carcinoma with a second group with similar tumor histological types and locations but with a negative family history of gastric cancer.

Materials and Methods

Patients and Tumors

Tumors from 65 gastric carcinoma patients who were operated upon in the Department of Surgery at the Technical University of Munich were analyzed. The patients were selected according to family histories of gastric cancer collected retrospectively or prospectively by standardized written questionnaires.

Group 1 consisted of 29 patients with a positive family history of gastric cancer and was further divided into three subgroups according to the strength of their family history. Group 1a was characterized by a strong family history of gastric cancer with nine patients having at least three affected first and/or second degree relatives and 2 patients having one first and one second degree relative affected with gastric cancer in one parental line. Group 1b had 10 patients with one first degree relative affected. Group 1c had eight patients with one second degree relative affected.

Group 2 consisted of 36 gastric carcinoma patients with a negative family history of gastric cancer, but consisted of similar proportions of histological types and tumor locations. Overall, tumors from 52 patients, which had been analyzed with eight microsatellite markers for MSI, have been included in previous studies.[19,26]

The site of the tumor was defined surgically according to the location of the main tumor mass and was divided into tumors located in the proximal, middle, and distal part of the stomach. Tumors arising in the proximal stomach included fundus and cardia carcinomas. Cardia carcinomas were defined as having more than 50% of the tumor mass located within the anatomic cardia (1-cm proximal and 2-cm distal to the Z line).

The tumors were staged according to the criteria of the UICC,[29] graded according to the WHO (World Health Organization),[30] and classified histologically according to the Laurén classification.[31] The detailed clinicopathological data of groups 1 and 2 are shown in Table 5.

Table 1. Microsatellite Loci Analyzed

Chromosome	Locus symbol	Genes	Repeat
1p31.1	BAT 40	*β-hydroxy-steroid-dehydrogenase*	(A)n
2p16-21	D2S123	*hMSH2*	(CA)n
2q	D2S71		(CA)n
3p25-26	D3S1317		(CA)n
4q11-13	BAT 25	*c-kit*	(A)n
5q11.2-q13.3	D5S107		(CA)n
5q21	D5S346	*APC*	(CA)n
6q27		*TBP*	(CAG)n
11q14	D11S901		(CA)n
17p12	D17S520	*Tp53*	(CA)n
17p12-p11.1	D17S261		(CA)n
18q12.2	D18S34	*DCC*	(CA)n

n, variable number of repeats.

DNA Isolation and Microsatellite Analysis

Paired nontumor and tumor DNA samples were isolated from the formalin-fixed, paraffin-embedded tissues from 58 cases after microdissection, as previously described.[19] DNA from seven cases was isolated from frozen tissue after phenol/chloroform extraction. Only tumor areas containing more than 50% tumor cells were used for DNA extraction.

Microsatellite analysis included 12 microsatellite markers, representing nine dinucleotide, two mononucleotide, and one trinucleotide repeats. The markers used are listed in Table 1. The polymerase chain reaction (PCR), gel electrophoresis, and PCR product detection methods have been previously described[19] with the exception that PCR was performed with fluorescent-labeled primers for markers D2S123 and D11S901 followed by analysis of the PCR products with an automated sequencing system (ABI 377, Perkin–Elmer, Branchburg, New Jersey). Overall, 54 cases were successfully amplified at all 12 loci, nine cases at 11 loci and two cases at 10 loci.

Scoring of Microsatellite Instability

An additional band in tumor DNA in comparison with normal DNA in a given locus was scored as MSI at that locus. A tumor was classified as having widespread MSI when at least half of the analyzed loci (≥6 loci) had MSI. Tumors having alterations in only a few loci (one to three loci) were classified as having a low level of MSI. Widespread MSI was confirmed by a second PCR for half of the markers showing alterations. MSI in the group with a low level was confirmed at least twice for all markers showing alterations.

Table 2. Fequency of Microsatellite Instability in Relation to the Family History

Family history*	Number of tumors tested	Number of tumors with microsatellite instability (%)		
		≥6 loci	1 to 3 loci	Total
Group 1	**29**	**5 (17%)†**	**12 (41%)‡**	**17 (59%)§**
Group 1a	11	3 (27%)	4 (36%)	7 (64%)
Group 1b	10	0 (0%)	5 (50%)	5 (50%)
Group 1c	8	2 (25%)	3 (38%)	5 (63%)
Group 2	**36**	**4 (11%)†**	**10 (28%)‡**	**14 (39%)§**

*Group 1a: at least three affected individuals with gastric cancer with first and/or second degree affected relatives; Group 1b: two affected individuals with first degree affected relatives; Group 1c: two affected individuals with second degree affected relatives; Group 2: no family history of gastric cancer in first and second degree relatives.

†‡§Not significant. †$P = 0.44$; ‡$P = 0.18$; §$P = 0.14$; Fisher's exact test (two-sided).

Scoring of Loss of Heterozygosity

We considered a clearly visible reduction in the relative signal intensity of one of the two alleles in the tumor DNA in comparison with the nontumor DNA, reflecting an allelic imbalance, to be LOH with the provision that, in some cases, allelic imbalance may be related to allelic amplification. Evaluation of LOH using fluorescent-labeled primers and analysis on the sequencing system for loci D2S123 and D11S901 was done essentially as described by Beckmann et al.[32] In brief, the ratio of the allele peak area was calculated for each normal and tumor sample after which the tumor ratio was divided by the normal ratio. If the calculated allele ratio was above 1.0 the ratio was converted to give a result in the range from 0.00 to 1.00. A tumor was considered to be positive for LOH if the allele peak ratio was equal or less than 0.6, representing an allelic signal reduction of at least 40%. Mononucleotide repeats seemed to be homozygote or ambiguous in respect to heterozygosity and were not evaluated for LOH. Tumors exhibiting MSI at a given locus were not evaluated for LOH.

Immunohistochemistry

Immunohistochemistry of the *p53* gene product was performed to analyze *p53* expression for an association with LOH at locus D17S520. Immunohistochemical analysis was performed on sections of formalin-fixed, paraffin-embedded tissue with the monoclonal antibody DO 7 (Dako, Hamburg, Germany) after microwave pretreatment for 2 × 5 minutes at 750 W. Staining was performed in a TechMate 500 staining machine (Dako) using the antibody in a dilution of 1:300 and the streptavidin-biotin-alkaline phosphatase system. Appropriate positive and negative controls were included. Insufficient tissue was available for immunohistochemical analysis in 4 of the 65 gastric carcinomas. The *p53* reaction was scored as positive for overexpression when a minimum of 10% of the tumor cells showed a nuclear stain.

Statistical Analysis

Statistical analysis was performed using Fisher's exact test (two-sided) or Pearson's χ^2 test (two-sided). A P value less than 0.05 was considered to be statistically significant.

Results

Microsatellite Instability

As for the frequency of MSI in group 1, MSI affecting at least one locus was observed in 17 of 29 (59%) tumors, among these were five (17%) cases with widespread MSI (≥6 loci) and 12 (41%) cases with MSI at a low level (one to three loci). The results of the subgroups are included in Table 2. In Group 2, 14 of 36 (39%) tumors showed MSI of at least one locus, including four tumors (11%) with widespread MSI and 10 (28%) with MSI at a low level. Statistical analysis was only performed by comparing results between groups 1 and 2 and revealed no statistically significant differences for MSI frequency. These results are summarized in Table 2.

In group 1, among the 12 tumors with a low rate of MSI, eight exhibited alterations at one locus, three at two and one at three loci, whereas in group 2, 9 of the 10 cases with a low rate of MSI had a single locus alteration with the remaining tumor showing alterations at two loci (Figure 1). No tumors with alterations at four or five loci were observed. Omitting the tumors with widespread MSI, a comparison of the two groups in respect to the mutation rate at the microsatellite markers used was calculated by dividing the total number of altered loci by the total number of microsatellite loci tested. In group 1, 17 of 281 (6%) analyzed microsatellite loci showed alterations compared

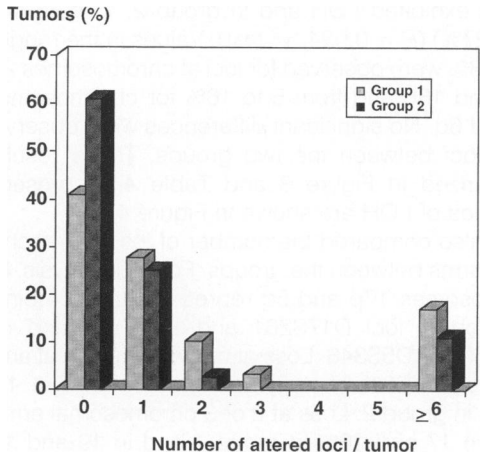

Figure 1. Comparison of the number of altered loci per tumor between groups 1 and 2.

Table 3. Incidence of Microsatellite Instability per Total Number of Performed Genotypes in Groups 1 and 2

Repeat type	Total number of alterations/ total number of microsatellites tested* (%) Group 1	Group 2	*P* value
Mononucleotide	4/48 (8.3)	4/64 (6.3)	0.723†
Dinucleotide	12/211 (5.7)	6/285 (2.1)	0.035‡
Trinucleotide	1/22 (4.5)	1/32 (3.1)	1.0†
Total	17/281 (6.0)	11/381 (2.9)	0.046‡

*Tumors with microsatellite instability ≥6 loci were excluded from this analysis.
†Fisher's exact test (two-sided).
‡χ^2 test (two-sided).

with 11 of 381 (2.9%) in group 2, a significantly higher mutation rate in group 1 as compared with group 2 (P = 0.046). As for the type of microsatellite marker, the difference was most prominent for dinucleotide repeats (5.7% *versus* 2.1%; P = 0.035). These results are summarized in Table 3.

Considering the pattern of MSI, tumors with widespread MSI showed predominantly a laddering pattern of expansions and contractions at di- and trinucleotide repeats, whereas the alterations at the mononucleotide repeats BAT 25 and BAT 40 were deletions in all but one case. The pattern of MSI typically seen in the tumors with a low rate of MSI consisted of distinct shifts of one to two repeat units with a strong preponderance of insertions over deletions (24 *versus* 3; 1 not classifiable). No significant differences in pattern were observed between the two groups. Representative examples of MSI are shown in Figure 2.

Loss of Heterozygosity

A comparison of the two groups for differences in rates of LOH showed a statistically significant difference at chromosome 17p12 (D17S520). In group 1, 13 of 22 (59%) tumors exhibited LOH and in group 2, 7 of 26 showed LOH (27%) (P = 0.024, χ^2 test). Values in the range from 14 to 28% were observed for loci at chromosomes 2p, 5q, 11q, and 18q and from 5 to 16% for chromosomes 2q, 3p, and 6q. No significant differences were observed for these loci between the two groups. These results are summarized in Figure 3 and Table 4. Representative examples of LOH are shown in Figure 4.

We also compared the number of losses per chromosomal arms between the groups. For this analysis, loss at chromosomes 17p and 5q represents the combined information of loci D17S261 and D17S520 and of loci D5S107 and D5S346. Loss at one chromosomal arm was observed in 28% of tumors in group 1 and in 19% of tumors in group 2. Loss at 2 or 3 chromosomal arms was found in 17 and 10% in group 1 and in 19 and 11% in group 2. Loss at 4 or 5 chromosomal arms was observed only in group 1 with rates of 4 and 7%, respectively.

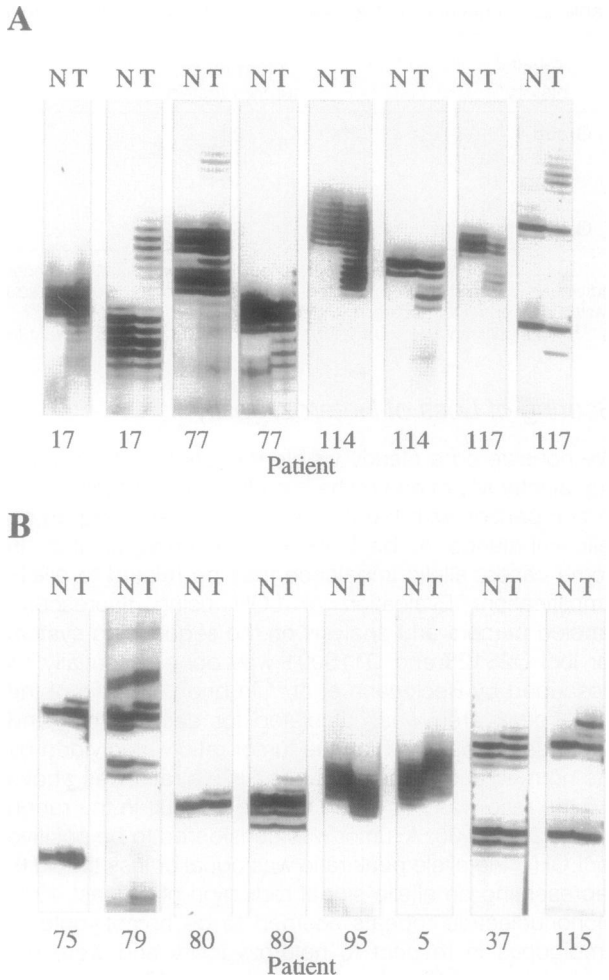

Figure 2. Examples of microsatellite instability. PCR products of paired nontumorous (N) and tumor (T) DNA from groups 1 and 2 are shown. **A:** Examples of widespread microsatellite instability characterized by the appearance of several additional bands in the tumor DNA. From **left** to **right:** Patient 17 (group 1) at BAT25; patient 17 at D5S107; patient 77 (group 1) at D5S107; patient 77 at D17S520; patient 114 (group 2) at BAT40; patient 114 at D5S107; patient 117 (group 2) at BAT 25; and patient 117 at D5S346. **B:** Examples of low level microsatellite instability characterized by the appearance of one or two additional bands in the tumor DNA. Group 1: patient 75 at 6q27; patient 79 at D2S71; patient 80 at D3S1317; patient 89 at D3S1317; and patient 95 at BAT40. Group 2: patient 5 at BAT40; patient 37 at D5S107; and patient 115 at 6q27.

p53 Immunohistochemistry

Immunostaining with the p53 antibody DO-7 revealed overexpression in 17 of 27 (63%) tumors in group 1 and in 19 of 34 (56%) tumors in group 2.

No correlation between LOH at locus D17S520 and *p53* overexpression was observed in either of the two groups. In group 1, 14 cases had *p53* overexpression by immunohistochemistry and were informative at locus D17S520. Nine of these (64%) showed LOH, whereas six cases were p53 immunonegative and informative, three of which (50%) exhibited LOH (P = 0.64). In group 2, 5 of 13 (38%) informative cases had *p53* overexpression and LOH *versus* 1 in 9 (11%) informative cases that were immunonegative and showed LOH (P = 0.178).

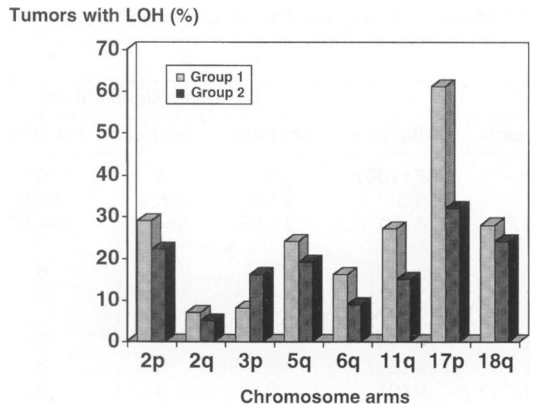

Figure 3. Comparison of chromosome arms exhibiting LOH between groups 1 and 2. LOH at chromosomes 17p and 5q represents the combined information of loci D17S261 and D17S520 and of loci D5S107 and D5S346 respectively.

Microsatellite Instability versus *Loss of Heterozygosity*

The tumors with a low rate of MSI were analyzed for an association with LOH on chromosome 17p12 (D17S520). In the study as a whole, among the 22 tumors with a low level of MSI, 21 were informative at locus D17S520, and among the 34 tumors negative for MSI, 26 were informative at this locus. Nine of 21 (43%) cases with low level MSI and informative for this locus showed LOH, whereas 11 of 25 (42%) cases in the whole study, which were negative for MSI and informative at this locus, exhibited LOH. This corresponded to 7 of 11 (64%) tumors with LOH at 17p12 and low level MSI *versus* 6 of 10 (60%) tumors with LOH and negative for MSI in group 1. In group 2, there were 2 of 10 (20%) positive tumors with LOH and low level MSI and 5 of 15 (33%) with LOH and negative for low level MSI, which was not statistically significant.

Table 4. Frequency of Loss of Heterozygosity in Gastric Carcinomas in Groups 1 and 2

Locus symbol	Chromosomal location	Tumors with LOH/informative tumors (%)	
		Group 1	Group 2
D2S123	2p16-21	5/17 (29)	4/18 (22)
D2S71	2q	1/14 (7)	1/20 (5)
D3S1317	3p25-26	1/12 (8)	3/19 (16)
D5S107	5q11.2-q13.3	5/20 (25)	2/23 (9)
D5S346	5q21	4/19 (21)	4/28 (14)
	5q*	6/25 (24)	6/31 (19)
TBP	6q27	3/19 (16)	2/22 (9)
D11S901	11q13	6/22 (27)	4/27 (15)
D17S261	17p12-p11.1	6/13 (46)	5/15 (33)
D17S520	17p12	13/22 (59)†	7/26 (27)†
	17p‡	14/23 (61)§	9/28 (32)§
D18S34	18q12.2	5/18 (28)	5/21 (24)

*Combined information of marker D5S107 and D5S346 for loss at chromosome 5q.
†Significantly different, $P = 0.024$, χ^2 test (two-sided).
‡Combined information of marker D17S261 and D17S520 for loss at chromosome 17p.
§Significantly different, $P = 0.040$, χ^2 test (two-sided).

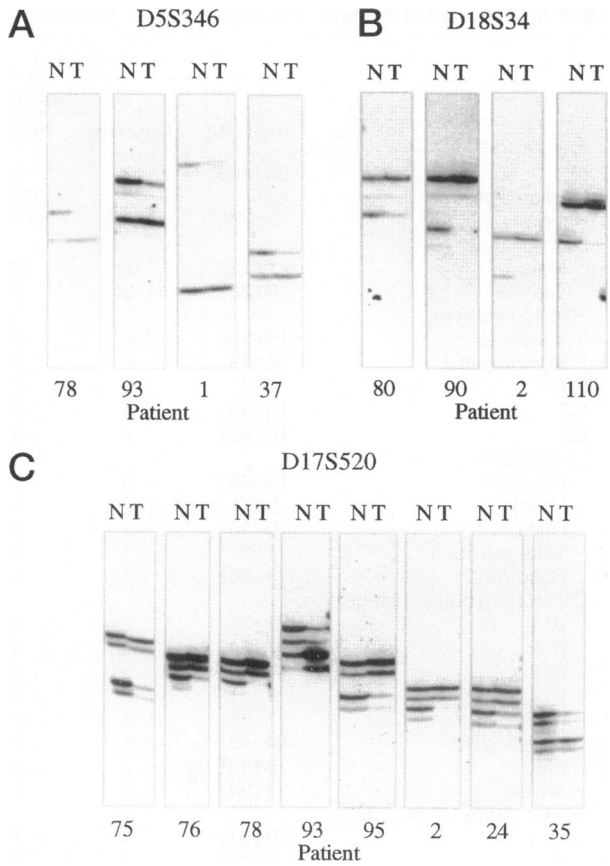

Figure 4. Examples of LOH. PCR products of nontumorous (**N**) and tumor (T) DNA of groups 1 and 2 are shown. LOH is demonstrated by a clearly visible reduction of the signal intensity of one of the alleles in the tumor DNA when compared with the nontumorous DNA. **A:** At locus D5S346, group 1, patients 78 and 93. Group 2, patients 1 and 37. **B:** At locus D18S34, group 1, patients 80 and 90. Group 2, patients 2 and 110. **C:** At locus D17S520, group 1, patients 75, 76, 78, 93, and 95. Group 2, patients 2, 24, and 35.

Microsatellite Instability and Loss of Heterozygosity versus *Clinicopathological Parameters*

We compared the tumors, which were negative for MSI, with those that had MSI at a low level and with those that had widespread MSI to various clinicopathological parameters including age, sex, histological type, tumor location, TNM status, and clinical stage. Comparisons were performed in the two groups and in the study as a whole. Table 5 shows the association of widespread MSI and low level MSI to the clinicopathological parameters in both groups. For statistical analysis, pathological and clinical tumor stages were divided into limited (pT1 + pT2 and I + II) and advanced stages (pT3 + pT4 and III + IV).

No significant correlation of widespread MSI was found in either group 1 or 2. Among the entire group of tumors, a statistically significant association was found between widespread MSI and female sex, with 25 males and 9 females negative for MSI and 3 males and 6 females with MSI ≥ 6 loci ($P = 0.046$). Widespread MSI showed a trend in association with more limited tumor stage (pT1 + pT2 *versus* pT3 + pT4; 21 *versus* 0%) ($P =$

Table 5. Association between Clinicopathological Features and Presence or Absence of Microsatellite Instability in Groups 1 and 2

	Group 1				Group 2			
		Microsatellite instability				Microsatellite instability		
	Total (%)	Negative	≥6 loci	1 to 3 loci	Total (%)	Negative	≥6 loci	1 to 3 loci
n	**29 (100)**	12	5	12	**36 (100)**	22	4	10
Median age	**67**	66	68	67	**65.5**	65.5	66.5	65.5
Range	**22–77**	22–77	57–71	48–72	**34–80**	34–80	46–77	34–77
Sex								
Male	**21 (72)**	9*	2*	10	**25 (69)**	16*	1*	8
Female	**8 (28)**	3*	3*	2	**11 (31)**	6*	3*	2
Histological type								
Intestinal	**15 (52)**	6	3	6	**16 (44)**	9	2	5
Nonintestinal	**14 (48)**	6	2	6	**20 (56)**	13	2	5
G1	**1 (3)**	1	0	0	**0 (0)**	0	0	0
G2	**13 (45)**	4	3	6	**4 (11)**	3	0	1
G3	**15 (52)**	7	2	6	**32 (89)**	19	4	9
Localization								
Proximal	**13 (45)**	5	2	6	**14 (39)**	7	2	5
Middle	**2 (7)**	2	0	0	**8 (22)**	7	0	1
Distal	**10 (35)**	3	3	4	**10 (28)**	6	2	2
Total stomach	**1 (3)**	1	0	0	**2 (6)**	1	0	1
Recurrent	**3 (10)**	1	0	2	**2 (6)**	1	0	1
Clinicopathological stage†								
pT1	**2 (7)**	2‡	0‡	0	**7 (19)**	6‡§	1‡	0§
pT2	**17 (54)**	4‡	4‡	9	**13 (36)**	8‡§	3‡	2§
pT3	**4 (14)**	3‡	0‡	1	**13 (36)**	7‡§	0‡	6§
pT4	**2 (7)**	2‡	0‡	0	**1 (3)**	0‡§	0‡	1§
pN0	**11 (38)**	4	1	6	**12 (33)**	10	1	1
pN1 + pN2	**14 (48)**	7	3	4	**22 (61)**	11	3	8
pM0	**18 (62)**	7	3	8	**25 (69)**	16	3	6
pM1	**7 (24)**	4	1	2	**9 (5)**	5	1	3
Tumor stage (UICC 1992)								
I	**9 (31)**	4	1	4	**12 (33)**	10	1	1
II	**5 (17)**	1	2	2	**5 (14)**	3	1	1
III	**4 (14)**	2	0	2	**9 (25)**	3	1	5
IV	**7 (24)**	4	1	2	**8 (22)**	5	1	2

*Significantly different in respect to the whole study; $P = 0.046$, Fisher's exact test (two-sided).
†Recurrent tumors not included. No information available for pT,N,M. Group 1: one tumor with MSI ≥6 loci.
‡Trend in an association combining T1 + T2 and T3 + T4 in the whole study. $P = 0.079$, Fisher's exact test (two-sided).
§Significantly different in Group 2 combining T1 + T2 and T3 + T4. $P = 0.046$, Fisher's exact test (two-sided).

0.079). We observed widespread MSI more frequently in tumors located in the distal part of the stomach when compared with tumors located in the middle and proximal part (25 *versus* 11%), but this difference was not statistically significant. Only slight variations were observed for the other clinicopathological parameters.

MSI at a low rate was significantly more frequent in tumors with more advanced stages in group 2 (pT1 + pT2 *versus* pT3 + pT4; 11 *versus* 50%; $P = 0.046$). MSI at a low rate was also observed more frequently, although not reaching statistical significance, in group 2 with tumors showing lymph node involvement (nodal negative versus nodal positive; 9 *versus* 42%; $P = 0.100$) and in tumors with more advanced clinical tumor stages (I + II *versus* III + IV; 12 *versus* 41%; $P = 0.109$). No such associations were observed in group 1. No statistically significant differences were observed for the other clinicopathological parameters in the two groups. In the study as a whole, no significant associations were observed.

Evaluation of an association of LOH with clinicopathological parameters was performed for marker D17S520 on chromosome 17p12 in the two groups as well as in the whole study and revealed no significant associations.

Discussion

In this study we compared the rates of MSI and LOH in gastric carcinomas from patients with and without a positive family history of the disease. MSI was seen either at multiple loci (≥6 loci) (widespread MSI) or only at a few loci (one to three loci) (low level MSI), representing a clear discontinuity in the number of altered loci per number of loci tested. Similar findings have been previously observed for gastric tumors[12,19–21,26,33] as well as for tumors of other organs.[34,35] Widespread MSI has been linked to defects in DNA mismatch repair genes,[9] but the origin of low level MSI is unknown. Hypotheses include background genetic tumor alteration or,[33] possibly, specific mutations in known mismatch repair genes resulting in an attenuated mutator phenotype[36] or mutations in, as yet, unidentified genes responsible for genetic stability.

Overall, group 1, consisting of patients with a variable degree of a positive family history of gastric cancer, had a high rate of low level MSI and LOH at chromosome 17p12. Specifically, low level MSI was seen in 41% of the tumors in group 1 and in 28% in group 2. The likelihood of detecting this type of MSI is dependent on the number

of microsatellite markers analyzed. This is illustrated by a previous study in which 138 microsatellite markers were used for 38 adenocarcinomas of the cardia with the result that 84% of the tumors exhibited low level MSI.[33] In order to accurately express low level MSI, the optimal method would be to express the rate of MSI as a ratio, i.e., total number of alterations per total number of microsatellite markers tested. For this calculation, we excluded the tumors with widespread MSI, because this is most likely caused by a different mechanism and is suspected to produce artificially high mutation rates.[37] In group 1, the mutation rate was 6% (17 in 281) *versus* 2.9% (11 in 381) in group 2 (2.1-fold higher). As for the type of repeat, dinucleotide repeats showed the greatest difference (2.7-fold). Microsatellite analysis in other studies of various tumor types have revealed MSI rates ranging from 0.2 to 5.2%, depending on the tumor type analyzed and the type of microsatellite markers used.[37] We also observed a striking preponderance of insertions over deletions, very reminiscent of the pattern for germ-line dinucleotide mutations,[38] which may reflect the inherent instability of these markers. However, this does not completely explain the higher frequency we observed in the tumors of patients with a familial background of the disease. Similar results have been reported in familial breast carcinoma patients in which the rate of MSI has been found to be significantly higher in familial *versus* nonfamilial cases.[39] Whereas a more prevalent contribution of exposure to exogenous toxins or inflammatory conditions to the higher rate of MSI in group 1 cannot be excluded, an alternative hypothesis is that this may be caused by subtle inherited differences in factors responsible for maintaining genetic stability. Support for this hypothesis comes from an analysis of MSI in lung carcinoma in which MSI similar to the type of low level MSI we observed in our study was significantly associated with rare H-*ras*1 VNTR alleles as well as rare alleles at another minisatellite loci.[40,41] This link between MSI and hereditary genetic markers led the authors to suggest that the same mechanism generates new alleles at minisatellite loci in the germ-line and produces MSI in tumors.

In a comparison of low level MSI in groups 1 and 2 with clinicopathological parameters, low level MSI in group 2 was significantly more frequent in advanced tumor stages (pT1 + pT2 *versus* pT3 + pT4; 11 *versus* 50%; $P = 0.046$). There was no such association in group 1, suggesting that low level MSI in this group occurs earlier in tumor progression and may have an association with tumor initiation in at least a subset of these patients. In line with this hypothesis are the results of a previous stage-dependent evaluation of MSI in gastric carcinoma with familial clustering revealing a higher incidence of MSI in early gastric cancer in a familial group compared with matched nonfamilial gastric cancer cases.[42] This MSI was, in the majority of cases, MSI corresponding to low level MSI.

LOH at locus D17S520 on chromosome 17p12 was the second most obvious difference between the two groups with 59% of the tumors showing LOH in group 1 and 27% in group 2 ($P = 0.024$). LOH of the short arm of chromosome 17 has been described in gastric carcinoma to range from 20 to over 60%.[43–45] Because the tumor suppressor gene *p53* is located near this chromosomal region,[46] we performed immunohistochemical analysis of the *p53* gene. A slight difference in overexpression was observed between the two study groups, as 63% of the tumors were positive in group 1 compared with 56% in group 2. The values in general are in the range of previous studies reporting an overexpression of *p53* in gastric cancers ranging from 23 to 58%.[47–49] However, the difference in the LOH pattern was not reflected by the immunohistochemical results. A lack of correlation between genetic alterations and the overexpression of the *p53* gene has been reported in various studies, including gastric cancer.[49,50] Thus our results may point to a functional difference leading to p53 protein accumulation in the two groups or to another gene located in this chromosomal region, which is more important in the carcinogenic process of tumors belonging to group 1. Furthermore, no association between LOH at 17p and low level MSI was seen, indicating that there is also no simple relationship between these two types of genetic alterations.

The rates of widespread MSI differed slightly between groups 1 and 2 (17 *versus* 11%). The rate of 11% in group 2 is within the range of 4 to 20% reported in previous European studies when comparing the number of tumors in these studies, which showed alterations in at least half of the loci tested.[14,20,24]

In group 1, consisting of patients having some familial background of the disease, widespread MSI was seen in 17% of the cases, indicating that it is not a marker per se of familial gastric carcinoma, at least with the broad criteria for a positive family history that we used. A previous study performed in Italy revealed a positive association of MSI comparable with widespread MSI with a positive family history of gastric cancer in first degree relatives.[25] If we had applied the same criteria to our study, our results would not have been substantially altered. In our study the most obvious association of a positive family history of gastric cancer was seen with low level MSI. Whether or not this difference may be explained by a variation of endogenous and/or exogenous factors in the study populations remains open. However, as we previously reported,[19,26] among five patients with widespread MSI and a family history, three patients showed a strong familial clustering of gastric cancer with at least three affected individuals, hinting that germ-line mutations in mismatch repair genes were involved in these particular families. Our mutation analysis of the *hMLH1*[26] and *hMSH2* genes (Keller G, Vogelsang H, Mueller J, Siewert JR, Höfler H, unpublished data) of these patients revealed one missense mutation in the *hMLH1* gene in a patient with one second degree relative with gastric cancer. Thus, the involvement of germ-line mutations in these particular families is still an unanswered question.

No significant differences in widespread MSI rates were seen in comparison with clinicopathological parameters either in group 1 or group 2. As a whole, widespread MSI was found significantly more frequently in females than in males. The reason for this association is unknown. We observed a preponderance of tumors lo-

cated in the distal part of the stomach exhibiting widespread MSI (25% in the distal part *versus* 15% in the proximal part), but our finding of 15% of positive cases with tumor location in the proximal stomach does not confirm the strong preference of widespread MSI and tumor location in the antrum, as has been suggested by others.[20,25] This discrepancy may be partially explained by the fact that in our study, we had a relatively high proportion of tumors located in the distal part of the stomach when compared with other studies.[20,25]

In conclusion, our data show that gastric carcinomas of patients having a positive family history of the disease are characterized by a higher rate of low level MSI, especially at dinucleotide repeats, and by a higher frequency of LOH on chromosome 17p12, findings that indicate that different genetic pathways are involved in the pathogenesis of gastric carcinomas arising in patients with and without a familial background of the disease.

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