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ALLEIC IMBALANCE IN GASTRIC CANCER : AN

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Allelic Imbalance in Gastric Cancer: An Affected Site on Chromosome Arm 3p

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In order to detect regions of DNA containing tumor suppressor genes involved in the development of gastric cancer, we performed an allelotype study on 78 gastric adenocarcinomas from a population composed largely of Texan Hispanics and Anglos, two ethnic groups that have a ratio of incidence rates of gastric cancer of approximately 2:1. In total, 42 microsatellite markers were employed, which detected at least one site per arm of each autosome in the human genome. These included several markers linked to known tumor suppressor genes (*TP53*, *APC*, *DCC*, *RB1*, and *BRCA1*). Sites showing quantitative allelic imbalance (AI) greater than 30% were located on 3p (36%), 11q (31%), 12q (38%), 13q (33%), 17p near *TP53* (74%), and 17q near *BRCA1* (32%). Among the 22% of cases showing microsatellite instability (MI), a subset (4 of 17) showed instability at 59% or more of sites tested. No ethnic bias was detected in cases showing MI or in cases with AI at sites with rates of AI above 30%. Tumors of the intestinal subtype were significantly more likely than diffuse tumors to show AI at D13S170 ($P = 0.01$). A deletion map of chromosome arm 3p was prepared for tumors with AI at D3S1478. These data indicate that a tumor suppressor gene on chromosome arm 3p is involved in the development of a subset of gastric cancers. *Genes Chromosom Cancer 13:263–271 (1995).* © 1995 Wiley-Liss, Inc.

INTRODUCTION

Gastric cancer is considered to be the second most common cancer worldwide (Parkin et al., 1988), affecting approximately 24,000 persons in the United States in 1994 (Boring et al., 1994). In recent years, progress has been made in discovering the genetic lesions underlying this cancer (for review, see Correa and Shiao, 1994). To examine the extent of tumor suppressor gene involvement in the origin of this disease, we performed an allelotype study of gastric cancers by screening for allelic imbalance (AI) at polymorphic sites. Similar studies on loss of heterozygosity (LOH) in other tumors have been effective in revealing sites of tumor suppressor genes (Lasko et al., 1991). In studies of gastric cancer, restriction fragment length polymorphism (RFLP) markers were previously employed to examine a small number of regions of the genome (Motomura et al., 1988; Wada et al., 1988; Fox et al., 1989; Neuman et al., 1991; Sano et al., 1991; Uchino et al., 1992; Ranzani et al., 1993). The advent of highly polymorphic microsatellite markers, now identified at relatively high density throughout the genome (NIH/CEPH Consortium, 1992; Weissenbach et al., 1992; Gyapay et al., 1994), permits analysis of formalin-fixed, paraffin-embedded archival specimens, greatly increasing the number of samples suitable for analysis. We report here on the results of an expanded AI analysis of gastric cancer that revealed involvement of multiple tumor suppressor genes, including a site on chromosome arm 3p.

MATERIALS AND METHODS

Patient Population

Formalin-fixed, paraffin-embedded surgical specimens of 67 gastric adenocarcinomas were obtained from six hospitals in San Antonio, Texas (University Hospital, Santa Rosa Medical Center Hospital, Northeast Baptist Hospital, Southwest Texas Methodist Hospital, St. Luke's Lutheran Hospital, and the Audie Murphy Veterans Administration Hospital), and from the Hospital General in Mexico City. An additional 11 frozen gastric cancers were obtained from the Bexar County Medical Center Hospital and from the Mayo Clinic. The protocol for acquisition of human tissues was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. Sixty-two percent of the patients were Hispanics, a minority group with an incidence of gastric cancer approximately twice that of Anglos in

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the state of Texas (Texas Dept. of Health, 1976–1985); 35% of the patients were Anglos, and 3% were African Americans. Hispanics in this study are predominantly of Latin American (mainly Mexican) descent, and Anglo patients are chiefly of northern European origin. The ethnicity of the patients and the site, grade, and subtype of the tumors were determined from medical records and from tumor registries in the respective hospitals. Tumors were staged by TNM criteria (Beahrs et al., 1992) and classified as intestinal (64% of cases) or diffuse subtype (36% of cases), according to Laurén (1965). Tumors intermediate in morphology (the "mixed" category) were included within the intestinal subtype.

From the tumors and from adjacent normal tissue, paraffin sections were harvested for AI analysis. We used the following precautions against cross-contamination of DNA from one patient's tumor to another: 1) Between blocks from each case, the microtome blades and all instruments were rinsed with 10% bleach and then with water and 2) single 10 μm sections were harvested in autoclaved microcentrifuge tubes or applied to glass slides for microdissection. Areas were selected that contained at least 50% of nuclei derived from malignant cells; therefore, tumors heavily infiltrated with inflammatory cells were excluded. Selected areas were scraped from the slide with a small piece of a razor blade. Sections from paraffin blocks containing yeast cells were harvested subsequently at the same microtome and used as negative controls in the polymerase chain reaction (PCR) for detection of any residual human DNA cross-contamination during microtomy and handling of tissue.

Sections were deparaffinized with octane and rinsed in 100% ethanol. Pellets were allowed to air dry and then were digested with 50–100 μl proteinase K (1 mg/ml, in 50 mM Tris-HCl buffer, pH 8, with 1 mM EDTA and 0.45% Tween-20) by overnight incubation at 52°C. The proteinase K was then denatured by heating at 95°C for 15 minutes. Templates were diluted 1:30 to 1:80 with autoclaved H_2O, and aliquots of 5 μl were employed in a PCR of 15 μl amplified in a Techne thermal cycler (model PHC-3).

AI Analysis

Tumors were screened for AI with primers that amplified 42 microsatellite markers, including at least one site at each arm of each autosome. In this study with PCR-based markers, we use the term AI instead of LOH, because we cannot exclude the possibility that we are detecting a gain instead of a loss of an allele. Markers were selected based on proximity to known tumor suppressor genes, or they were selected on each chromosome arm from a series of markers with high rates of heterozygosity. Markers were chosen to have rates of heterozygosity as high as possible; the markers listed in Figure 1 had an average percentage of heterozygosity of 81%. However, the calculated rate of heterozygosity in this study was less than expected (on average, 67%) compared to reported rates. This is because, in the absence of family studies, we conservatively classified alleles as heterozygous only if an unambiguous discrimination between alleles could be made. The microsatellite markers used are listed in Figure 1.

TABLE 1. Variety in Tumors of Frequency of Sites Exhibiting Microsatellite Instability[a]

No. of cases	No. of sites with MI	No. of sites tested	Percentage of sites with MI (of total examined)
59	0	8–42	0
7	1	22–35	3–5
2	2	27, 30	7
1	3	32	9
3	4	32–41	10–13
1	13	22	59
1	15	28	54
1	24	31	77
1	24	33	73

[a]For each tumor, the number of sites exhibiting MI is compared to the total number of sites examined. Two tumors tested at fewer than eight sites were omitted in this analysis. Although most cases with MI were affected at fewer than 13% of sites tested, four tumors showed extreme genetic instability.

One primer of each pair was labeled with polynucleotide kinase and γ-^{32}P-ATP. We used the primers to amplify DNA prepared as described above from a tumor and its matched normal tissue control for each patient in the study. PCR mixtures were assembled by use of a multichannel pipettor in 96- well plates that fit directly into the thermal cycler. PCR mixtures contained 100 nM in each primer and 200 μM in each dinucleotide triphosphate. $MgCl_2$ concentrations ranged from 1.5 to 2.5 mM. Spermidine (1 mM) was added to some PCR reactions to facilitate amplification of DNA from paraffin-embedded tissues. Annealing temperatures varied with each primer, from 52°C to 62°C, and amplifications were carried out for 35 cycles. PCR products were mixed with a denaturing dye and heated for 5 minutes at 95°C before being applied to gels.

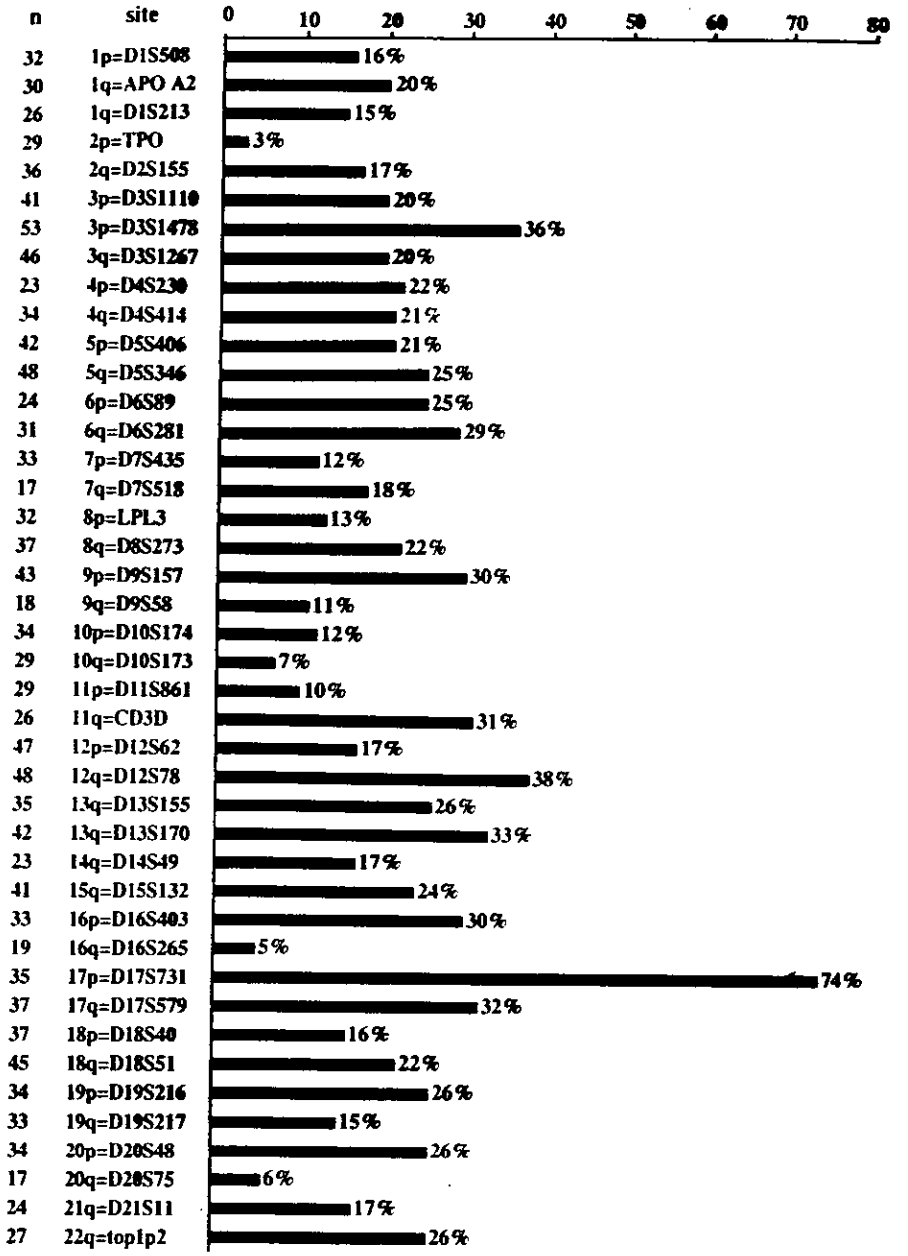

Figure 1. Summary of allelic imbalance (AI) analysis. Bars represent rates of AI measured at sites on each autosome. Several chromosome arms were evaluated with more than one marker.

For detection of heterozygosity, PCR products were electrophoresed on formamide-urea denaturing gels (7% polyacrylamide, 34% urea, 32% formamide) for 3–5 hours at 60 W for separation of the DNA fragments. Gels were fixed in 10% acetic acid/10% methanol and dried under vacuum at 80°C. Dried gels were analyzed by autoradiography on Kodak X-Omat AR film. Informative results were those in which two major bands were evident, indicating that the individual had inherited two different alleles at that locus. The relative intensity of bands was determined both by visual inspection and by quantitation of dried gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Using the PhosphorImager, we calculated an imbalance factor, which was the ratio of allele intensities in the tumor relative to the ratio of allele intensities in the normal tissue. Tumors that had an average imbalance factor greater than 1.3 were classified as having AI at that locus. Assuming a maximum of 50% contaminating normal cells in the tumors studied and setting an imbalance factor of 1.3 as a criterion for loss places tumors that were heterogeneous in their allele loss (up to 50% heterogeneity within the tumor) in the imbalanced category (Gruis et al., 1993). Evaluations based on the dividing point of 1.3 agreed well with evaluations made by visual inspection. Each analysis was performed at least twice to ensure reproducible detection of AI.

Statistical Analysis

Associations between categorical variables were tested by use of contingency tables. Two-by-two tables were examined with Fisher's exact test. We used analysis of variance (ANOVA) to compare mean stage and grade for informative cases with and without AI at D3S1478, CD3D, D12S78, D13S170, D17S731, and D17S579, adjusting for gender, ethnicity, and tumor subtype (intestinal or diffuse). We used residual diagnostics to verify the validity of this analysis. Grade and stage were converted to numerical values for analysis as follows: well differentiated, 1; moderately differentiated, 2; poorly differentiated, 3; and undifferentiated, 4: stage 1A, 1.0; stage 1B, 1.5; stage II, 2.0; stage IIIA, 3.0; stage IIIB, 3.5; and stage IV, 4.0. We used Spearman's correlation coefficients to test for associations between the number of sites with microsatellite instability (MI) and continuous variables such as stage and grade. Because the number of sites with MI was skewed in distribution, the Savage nonparametric test (optimal for exponentially skewed distributions) was used to compare subgroups of tumors. Bartholomew's test for trend was used to compare subtype frequencies to extent of MI. Analyses were performed with the SAS statistical software (SAS Institute, Cary, NC).

RESULTS

Using the primers listed in Figure 1, we successfully amplified DNA from routinely processed, paraffin-embedded tumor specimens, including tissue that had been in paraffin since 1975, which was the oldest tissue analyzed. The rates of success with amplification varied with the primers used; not every template was amplified successfully with every primer pair. The percentages of AI quantitated by the PhosphorImager and the numbers of informative cases at each site in our initial screening for affected sites are shown in Figure 1. Sites showing the highest rates of AI were located on chromosome arms 3p, 11q, 12q, 13q, 17p, and 17q at D3S1478, CD3D, D12S78, D13S170, D17S731, and D17S579, respectively. Examples of AI analyses and their quantitation are presented in Figure 2. The site of AI on 3p was examined in more detail. We wished to identify the smallest common deletion near the D3S1478 marker; therefore, we analyzed the tumors showing LOH at D3S1478 for their status at additional markers on chromosome arm 3p. The results of this analysis for tumors showing partial loss of chromosome arm 3p are shown in Figure 3.

When patients whose tumors exhibited AI at any of the six sites with the highest rates of AI were

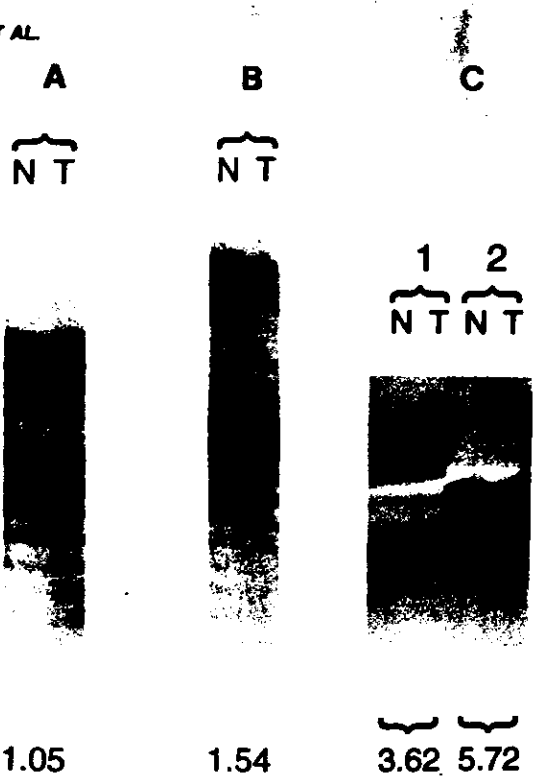

Figure 2. Example of analysis and quantitation of AI. Lanes show fragments amplified from normal (N) and tumor (T) DNA from four different patients. Values of individual allelic imbalance factors (IF) are printed below each analysis. **A:** The samples amplified at D3S1478 show an IF of 1.05; alleles are balanced in normal and tumor DNA, and heterozygosity is retained. **B:** Another patient's DNA amplified at D3S1478 shows AI, with an IF of 1.54. PhosphorImager readings from analysis of this tumor were significantly different from those of the normal tissue ($P = 0.0014$, t test; $n = 7$ independent experiments). **C:** Cases 1 and 2 were amplified at marker D12S309 and show extreme AI with IFs of 3.62 and 5.72. Typically, in tumors showing AI, one of the sets of bands is reduced in intensity but is not completely lost due to contamination of the tumor by normal cells or to heterogeneity in the tumor.

Using the PhosphorImager, we calculated an imbalance factor, which was the ratio of allele intensities in the tumor relative to the ratio of allele intensities in the normal tissue. Tumors that had an average imbalance factor greater than 1.3 were classified as having AI at that locus. Assuming a maximum of 50% contaminating normal cells in the tumors studied and setting an imbalance factor of 1.3 as a criterion for loss places tumors that were heterogeneous in their allele loss (up to 50% heterogeneity within the tumor) in the imbalanced category (Gruis et al., 1993). Evaluations based on the dividing point of 1.3 agreed well with evaluations made by visual inspection. Each analysis was performed at least twice to ensure reproducible detection of AI.

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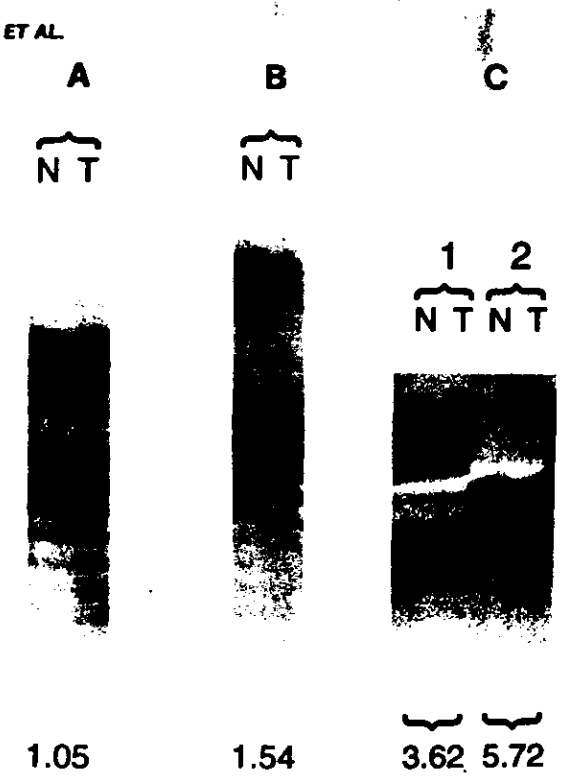

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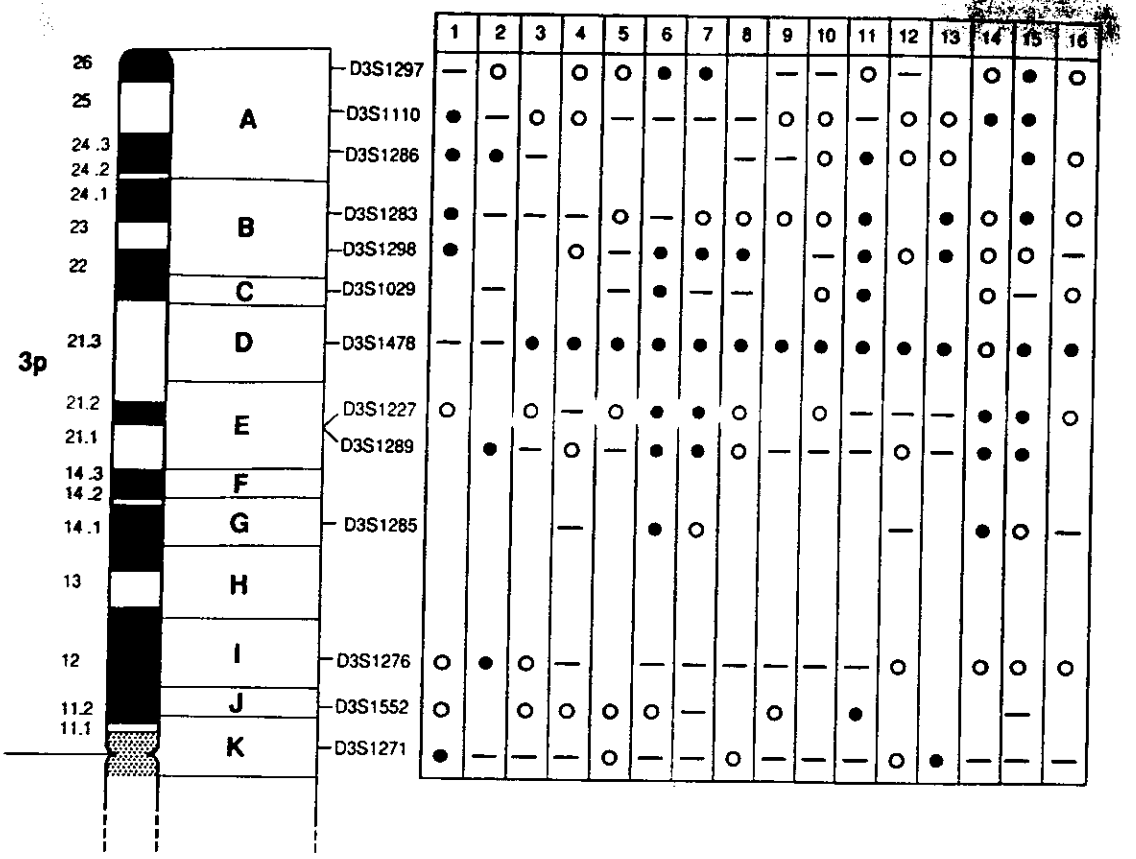

Figure 3. Map of chromosome arm 3p showing tumors with AI on part of 3p. Solid circle, marker informative and showing AI in the tumor compared to the normal DNA; open circle, marker informative with no AI; dash, marker noninformative; blank space, no data. Map order follows O'Connell et al. (1994). Divisions of chromosome 3 follow Leach et al. (1994).

analyzed by ethnic origin (Hispanic vs. Anglo), no significant differences from cases that retained allelic balance at these sites were detected ($P > 0.2$ in each case). The presence of AI at the six sites was not associated with stage, grade, or subtype of the tumor, except that a higher fraction of tumors of the intestinal subtype showed AI at D13S170 compared to diffuse tumors (11% vs. 50%; $P = 0.01$).

The site most frequently affected (lost in 26 of 35 informative cases, or 74%) was D17S731, which is closely linked to the *TP53* gene (P. O'Connell and R. Leach, unpublished data). Other markers that we tested were linked to the *DCC* gene on chromosome arm 18q (Fearon et al., 1990) and a site mapped to 8p22 linked to prostate (MacGrogan et al., 1994) and colorectal cancer (Cunningham et al., 1993). In our study, these sites showed AI at relatively low rates (22% and 13%, respectively).

In a subset of gastric cancers we detected MI, which was revealed by the presence of bands of altered sizes in the analysis of the tumor compared to that of the benign cells (Fig. 4). A wide variety of changes in the electrophoretic mobility of bands was observed. MI occurred at multiple sites in ten of the cases (13%) and at single sites in seven other cases (9%). Table 1 shows the wide range of the number of sites affected in the tumors. A striking finding was that tumors with MI fell into two groups: those with MI at four or fewer sites (13% or less) and those with MI at 13 or more sites (59% or more of sites examined). The latter group also showed more extreme alterations in the size of amplified fragments. Cases exhibiting MI (22% of the total) did not differ from those without MI in stage or subtype ($P > 0.2$) or by the gender or ethnicity of the patient ($P > 0.38$ in all cases). However, grouping tumors into categories of cases with no MI (60% intestinal subtype), cases with one to four sites affected (69%), and cases with 13 or more sites affected (100%) revealed a nonsignificant association ($P = 0.10$) of extent of MI and subtype. We also noted a nonsignificant trend for samples with MI from older patients (mean age for MI

Figure 4. Microsatellite instability. Two gastric cancers demonstrate MI of varying degrees at marker D13S170. The tumor from the case labeled 1 shows a large increase in size in the fragments amplified. Such patterns were commonly observed in the subset of four tumors that showed rates of affected sites greater than 50%. The tumor from the case labeled 2 shows only slightly altered mobility in bands amplified from the tumor compared to the bands in its benign control. All of the normal and tumor pairs from Figures 2 and 4 are from different patients.

cases, 70.8; others, 64.1; $P = 0.063$). AI at D3S1478 was not associated with MI ($P = 0.53$).

DISCUSSION

In this study, we detected elevated rates of AI at several sites in the genome. One site, which was lost in 36% of informative cases, was on chromosome arm 3p at marker D3S1478. Cytogenetic studies have previously detected breakpoints at 3p in gastric cancers (Ochi et al., 1986; Seruca et al., 1993). Several tumor suppressor genes have been identified on 3p. One of these is associated with the Von Hippel-Lindau disease; we detected AI at relatively low levels (16%) using marker D3S1110, which is closely linked to this gene (Crossey et al., 1993). Another tumor suppressor gene, located at 3p21.3-p21.2 (Daly et al., 1993), is affected in lung tumors (Naylor et al., 1987). Mutations in this gene may also occur in cervical (Yokota et al., 1989), anal (Muleris et al., 1987), and renal cell (Zbar et al., 1987) carcinoma. Because D3S1478 is close to a gene implicated in lung tumors, it is possible that this tumor suppressor gene is affected in these gastric cancers. However, until the gene affected in lung cancer has been identified and sequenced in gastric cancers, it will be impossible to tell whether that gene or some other was affected in the tumors we analyzed.

We found elevated rates of AI at marker D12S78 located at 12q14-q24.33. Murty et al. (1992) observed two sites of LOH in male germ cell tumors at 12q13 and 12q22, and they postulated that two different tumor suppressor genes on chromosome arm 12q are involved in the origin of those cancers. From the location of our site of AI at D12S78, it is possible that one of the tumor suppressor genes involved in the genesis of germ cell tumors is also affected in some gastric cancers. Fey et al. (1989) reported elevated rates of LOH in gastric cancers at a marker mapped to 12q24-qter; their study and ours may reflect the loss of the same tumor suppressor gene.

We also examined several sites tightly linked to other known tumor suppressor genes. One of these, D17S731, is closely linked to *TP53*. The involvement of *TP53* in gastric cancer has been well established (Kim et al., 1991; Tamura et al., 1991). Our finding of 74% AI is consistent with results of assays for LOH at sites near *TP53* obtained in gastric cancers in other populations (Fey et al., 1989, 83%; Sano et al., 1991, 68%). This result demonstrates that our analysis of paraffin-embedded specimens with use of microsatellite markers can readily reveal high rates of AI where they are known to exist.

The genetic instability evident as MI may occur as a result of a mechanism of tumorigenesis that is independent of the action of tumor suppressor genes or oncogenes (Aaltonen et al., 1993; Ionov et al., 1993; Thibodeau et al., 1993). MI may result from germline or somatic mutations in genes that control mismatch repair, such as the *hMSH2* (Fishel et al., 1993; Leach et al., 1993) or *hMLH1* gene (Bronner et al., 1994; Papadopoulos et al., 1994). We noted an apparent dichotomy in MI in the cases we studied. Although most of the cases were minimally affected, a few of them showed large changes in length of the banding patterns and had a high frequency of sites affected. MI in colon cancers has been described as falling into two groups differing by the extent of variation in fragment size. The two groups were called type I and type II by Thibodeau et al. (1993). The reason for the dichotomy of the grouping of gastric tumors

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DISCUSSION

In this study, we detected elevated rates of AI at several sites in the genome. One site, which was lost in 36% of informative cases, was on chromosome arm 3p at marker D3S1478. Cytogenetic studies have previously detected breakpoints at 3p in gastric cancers (Ochi et al., 1986; Seruca et al., 1993). Several tumor suppressor genes have been identified on 3p. One of these is associated with the Von Hippel-Lindau disease; we detected AI at relatively low levels (16%) using marker D3S1110, which is closely linked to this gene (Crossey et al., 1993). Another tumor suppressor gene, located at 3p21.3-p21.2 (Daly et al., 1993), is affected in lung tumors (Naylor et al., 1987). Mutations in this gene may also occur in cervical (Yokota et al., 1989), anal (Muleris et al., 1987), and renal cell (Zbar et al., 1987) carcinoma. Because D3S1478 is close to a gene implicated in lung tumors, it is possible that this tumor suppressor gene is affected in these gastric cancers. However, until the gene affected in lung cancer has been identified and sequenced in gastric cancers, it will be impossible to tell whether that gene or some other was affected in the tumors we analyzed.

We found elevated rates of AI at marker D12S78 located at 12q14-q24.33. Murty et al. (1992) observed two sites of LOH in male germ cell tumors at 12q13 and 12q22, and they postulated that two different tumor suppressor genes on chromosome arm 12q are involved in the origin of those cancers. From the location of our site of AI at D12S78, it is possible that one of the tumor suppressor genes involved in the genesis of germ cell tumors is also affected in some gastric cancers. Fey et al. (1989) reported elevated rates of LOH in gastric cancers at a marker mapped to 12q24-qter; their study and ours may reflect the loss of the same tumor suppressor gene.

We also examined several sites tightly linked to other known tumor suppressor genes. One of these, D17S731, is closely linked to *TP53*. The involvement of *TP53* in gastric cancer has been well established (Kim et al., 1991; Tamura et al., 1991). Our finding of 74% AI is consistent with results of assays for LOH at sites near *TP53* obtained in gastric cancers in other populations (Fey et al., 1989, 83%; Sano et al., 1991, 68%). This result demonstrates that our analysis of paraffin-embedded specimens with use of microsatellite markers can readily reveal high rates of AI where they are known to exist.

The genetic instability evident as MI may occur as a result of a mechanism of tumorigenesis that is independent of the action of tumor suppressor genes or oncogenes (Aaltonen et al., 1993; Ionov et al., 1993; Thibodeau et al., 1993). MI may result from germline or somatic mutations in genes that control mismatch repair, such as the *hMSH2* (Fishel et al., 1993; Leach et al., 1993) or *hMLH1* gene (Bronner et al., 1994; Papadopoulos et al., 1994). We noted an apparent dichotomy in MI in the cases we studied. Although most of the cases were minimally affected, a few of them showed large changes in length of the banding patterns and had a high frequency of sites affected. MI in colon cancers has been described as falling into two groups differing by the extent of variation in fragment size. The two groups were called type I and type II by Thibodeau et al. (1993). The reason for the dichotomy of the grouping of gastric tumors

showing MI is still unclear. Understanding of this phenomenon awaits investigation of the specific genetic lesions occurring in the responsible mismatch repair genes.

It has been proposed that *TP53* mutations and mutations in mismatch repair genes leading to MI occur in different populations of gastric cancers, each manifesting a different form of genetic instability that contributes to tumor progression (Strickler et al., 1994). We found no evidence of such a dichotomy, because MI and AI near *TP53* frequently affected the same tumors. There was no significant association between cases showing AI at D17S731 (the marker we used that was most closely linked to *TP53*) and the presence or absence of MI ($P = 0.153$). In addition, in a previous study (Schneider et al., 1994), the gastric cancers analyzed here were analyzed by immunohistochemistry (IHC) for accumulation of p53 protein. This protein accumulates as a result of many common missense mutations in the *TP53* gene. In that study, 62% of total cases (44 of 71 tested here) showed accumulated (abnormal) p53 protein, suggesting a mutation in the *TP53* gene. Among the 44 p53-positive cases, 27% exhibited MI. The p53 status by IHC and the presence or absence of MI were not associated ($P = 0.635$). The four cases with more than 50% of sites showing MI were all positive for p53 by IHC.

Previous LOH studies of gastric cancer in other patient populations with the use of RFLP markers showed deletions at 1q, 5q, 7p, 7q, and 17p (Sano et al., 1991); 1q and 12q (Fey et al., 1989); 17p and 18q (Uchino et al., 1992); 13q (Motomura et al., 1988); 5q, 11p, 17p, and 18q (Ranzani et al., 1993); and 5q (Neuman et al., 1991). Sano et al. (1991) reported elevated rates of loss at 1q21-24 at marker D1S7, and Fey et al. (1989) reported LOH at *MUC1* mapped to 1q21-24. We found 20% AI at marker *APO A2* mapped to 1q21-23. Elevated rates of LOH were reported at markers D7S21 (7p22-ter) and D7S22 (7q36-ter; Sano et al., 1991), but our markers at 7p15.1-p22 (D7S435) and 7q21-q31 (D7S518) showed only modest rates of AI of 12% for 7p and 18% for 7q. Using several markers at 13q22, Motomura et al. (1988) found LOH in 41% of gastric cancers. We also found an elevated rate of AI of 33% at marker D13S170 mapped to 13q22-q31. We found a relatively low rate of AI (22%) at D18S51, a locus that is linked to the *DCC* gene. Our finding of a lower rate of affected cases than that in a previous study (greater than 50% LOH for several 18q markers; Uchino et al., 1992) may result from differences in the markers employed, in patient populations, or in variability due to sample sizes.

The *APC* and *MCC* loci lost heterozygosity in more than 80% of cases in a study of gastric cancer cells selected by cell sorting for aneuploidy, whereas studies on unsorted tumor cells generated lower rates of LOH (Tamura et al., 1993). From a screen for mutations of the *APC* gene in gastric cancer, Horii et al. (1992) reported a low rate of detected mutations (7%, or 3 of 44 cases tested). Two of the three mutations reported in that study were accompanied by LOH. Sano et al. (1991) found that 42% of well differentiated stomach cancers lost heterozygosity at D5S43, a marker linked to *APC*. We tested a different marker tightly linked to the *APC* gene, D5S346 (Spirio et al., 1991), and found AI in 27%. Tamura et al. (1993) suggested that failure to find high rates of LOH at sites linked to the *APC* gene is due to contamination by benign cells; however, this does not apply to our specimens, which contain a sufficiently high proportion of tumor cells to reveal 74% AI at a site near *TP53*. Tamura et al. (1993) used only aneuploid tumors, and, consequently, their analysis applies to a subset of gastric cancers. Saal et al. (1993) reported that intestinal-type tumors are likely to be aneuploid, whereas diffuse tumors are primarily diploid. In our study, nine of ten cases showing AI at D5S346 were intestinal-type cases, although this association was not significant ($P = 0.16$). Our study and that of Tamura et al. (1993) suggest an increased likelihood of 5q21 losses in intestinal-type tumors, assuming that aneuploid tumors are primarily of the intestinal type. However, another study with a small number of tumors showed a greater rate of LOH near *APC* in diffuse-type tumors than in intestinal-type tumors (McKie et al., 1993). The type and favored order of genetic lesions occurring in the development of the diffuse type of tumor are apparently different from those in the intestinal type (Correa, 1988). Future investigations into the genetics of the two subtypes of gastric cancer will be useful in clarifying these differences.

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