Genetic Tumor Markers With Prognostic Impact in Dukes' Stages B and C Colorectal Cancer Patients

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Purpose: **To examine several genetic changes in primary colorectal carcinomas (CRCs) from patients with 10 years of follow-up and associate the findings with clinicopathologic variables.**

Material and Methods: **DNA from 220 CRCs were analyzed for allelic imbalances at 12 loci on chromosome arms 1p, 14q, 17p, 18q, and 20q, and the microsatellite instability (MSI) status was determined. The clinical significance of the tumor protein 53 (*TP53*) mutations was re-evaluated.**

Results: **Patients with tumors containing 17p or 18q deletions had shorter survival than those without these alterations ($P = .021$, $P = .008$, respectively). This was also significant for the Dukes' B group ($P = .025$, $P = .010$, respectively). Furthermore, patients with tumors showing losses of both chromosome arms revealed an even poorer disease outcome than those with either 17p or 18q loss. Patients with low increase in 20q copy number in their tumors had longer survival compared with those without changes ($P = .009$) or those with a high increase of copy number ($P = .037$). This was also evident for the Dukes' C group ($P = .018$, $P = .030$, respectively). MSI was seemingly a beneficial marker for survival ($P = .071$). A significant association between mutations affecting the L3 zinc-binding domain of *TP53* and survival was confirmed in this cohort after 10 years of follow-up, and also was found to apply for patients in the Dukes' B group. Several associations were found among genetic and pathologic data.**

Conclusion: **The present study indicates that 17p, 18q, and 20q genotypes, and *TP53* mutation status add information in the subclassification of Dukes' B and C patients and may have impact on the choice of treatment.**

J Clin Oncol 21:820-829.

COLORECTAL CANCER (CRC) is one of the most common causes of cancer-related death in Norway, as it is in most of the Western world, and despite major advances in the diagnosis and treatment of this disease, mortality has remained unchanged during the last 20 years.[1,2] Currently, Dukes' classification, or one of its many modifications, is the most commonly used predictor of prognosis for CRC patients. Thirty percent to 40% of Dukes' stages B and C patients will experience relapse and die of the disease.[1,2] Therefore, identification of additional prognostic markers to supplement standard clinical and pathologic staging could potentially subdivide patients in Dukes' stages B and/or C into groups with high and low risk of relapse after surgery, and thus have impact on the choice of treatment.

A minor subgroup of sporadic primary CRCs display microsatellite instability (MSI),[3-6] a result of a defective DNA mismatch repair,[7] but the majority of the carcinomas harbor numerous chromosome aberrations, and chromosomal instability seems to be quite pronounced in this tumor type.[8]

Although numerous studies have reported MSI in series of CRC, uncertainty remains whether positive MSI status is associated with prolonged survival for the patient.[4,5,9-21] Deletions of the whole or part of chromosome arm 1p is found in early as well as in advanced stages of colorectal tumorigenesis.[22-25] The smallest region of common deleted sequences of 1p is 1p32-pter,[25-31] a region demonstrated to have functional importance in colorectal tumorigenesis.[32] The target gene(s) for the reported deletions is unknown. Monosomy of chromosome 14 as well as allelic imbalance (AI) at 14q have been found both in primary and in advanced CRCs.[23,24,33,34] Deletions at 17p and 18q are two of the most common changes in CRCs[22,35-37] and often reflect inactivation of tumor protein 53 (*TP53*) at 17p13,[38] and deleted in colorectal carcinoma (*DCC*)[39] and mothers against decapentaplegic homolog 2, 4 (*SMAD2, 4*)[40,41] at 18q21. Several studies have shown associations between deletion of 17p or 18q and reduced survival for patients with CRC,[12,16,42-53] whereas other studies have not.[9,45,52,54-56] The significance of TP53 for the clinical outcome of CRC is controversial, with reports of both favorable and unfavorable results in patients with overexpressed or mutated *TP53*.[44,57,58] Gain of several chromosome regions that may pinpoint amplified oncogenes has been reported.[26] In a large multicenter study of CRCs, mutations in the oncogene *K-RAS* were found associated with poor survival rate.[59,60] Some studies have reported that gain of chromosome arm 20q is present more often in liver metastases from CRC patients than in primary carcinomas.[61-66]

We have analyzed several selected markers in a consecutive series of 220 CRCs in relation to clinical data after 10 years of patient follow-up. In addition, the new data have been compared with previously reported genetic data in the same series, and the impact of *TP53* mutations status on survival after 10 years has been re-evaluated.

MATERIALS AND METHODS

Patients and Samples

Two-hundred twenty tumor and corresponding blood samples from a consecutive series of CRC patients prospectively collected from seven

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Submitted May 29, 2002; accepted November 8, 2002.

Supported by the Norwegian Foundation for Health and Rehabilitation (C.B.D.) and the Norwegian Cancer Society (L.T., R.A.L.).

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Table 1. Associations Between Genotype Changes at Chromosome Arms 1p, 14q, 18q, 20q, and Microsatellite Status and Clinicopathologic Variables in Colorectal Cancer Patients

	1p (n = 184)*		14q (n = 201)*		18q (n = 184)*		20q (n = 154)*				Microsatellite Status (n = 275)†	
	AI‡	P‖	AI‡	P‖	AI‡	P‖	Gain‡	P‖	Ampl‡	P‖	MSI§	P‖
Sex		.086		NS		NS		NS		NS		NS
Male	39/60		37/70		71/30		28/22		27/22		15/127	
Female	45/40		41/53		58/25		25/25		27/25		21/112	
Age, years		NS		NS		NS		.071		.089		.057
< 71	46/49		44/57		59/32		22/28		23/28		23/112	
≥ 71	38/51		34/66		70/23		31/19		31/19		13/127	
Location		NS		NS		.069		NS		NS		< .001
Right colon	21/33		19/37		30/22		16/15		12/15		25/64	
Left colon	18/25		18/30		34/11		11/13		16/13		7/61	
Rectum	45/42		41/56		65/22		26/19		26/19		4/114	
Histologic grade		NS		NS		NS		NS		NS		< .001
Poorly differentiated	11/15		8/20		18/8		5/6		8/6		15/20	
Moderately differentiated	68/79		65/96		102/45		45/37		43/37		20/204	
Well differentiated	5/6		5/7		6/5		3/4		3/4		1/15	
Dukes' classification		NS		NS		NS		NS		NS		NS
A	12/13		11/18		18/9		10/6		6/6		5/37	
B	37/42		39/49		59/21		25/17		27/17		17/107	
C	25/33		22/41		36/22		13/19		16/19		10/70	
D	10/12		6/15		16/3		5/5		5/5		4/25	

Abbreviations: AI, allelic imbalance; MSI, microsatellite instability; MSS, microsatellite stable; NS, not significant.

*Thirty-six tumors (1p), 19 tumors (14q), 36 tumors (18q), and 35 tumors (20q) were noninformative. Seven tumors showed deletions (20q), and 24 showed both gain and deletion (20q). These tumors were excluded from further statistical analyses.

†Data from Lothe et al,[4] Thorstensen et al,[67] and the present study.

‡AI: numbers of tumors with allalic imbalance/number of tumors with retained heterozygosity; Gain: number of tumors with less than three times increase in copy number/number of tumors with retained heterozygosity; Ampl: number of tumors with three times or more increases in copy number (amplification)/number of tumors with retained heterozygosity.

§Number of tumors with MSI/number of tumors with MSS.

‖Comparison of different groups were tested with Pearson's χ^2 test or Fisher exact test, *P* values are two sided and are considered statistically significant when $P < .05$ and not significant when $P > .10$.

hospitals in the Oslo region between 1987 and 1989 were included in this study. Clinical data and pathologic characteristics of the tumors are given in Tables 1 and 2. The series consisted of 114 males and 106 females; the median age at diagnosis was 71 years (range, 26 to 94 years) for males and 69 years (range, 41 to 92 years) for females. The median survival time for these patients was 84.0 months (range, 0.5 to 147.2 months) and was established as time between surgery and death or the last update (July 1, 1999). Cause-specific deaths have been recorded from hospital and postmortem report. All of the patients underwent surgery alone as the curative treatment except for a few patients with rectal tumors who also received postoperative radiation therapy. At the time of relapse, approximately one third of the patients received palliative chemotherapy treatment. This study

Table 2. Relation Between Genotype Changes at 1p, 14q, 18q, and 20q and Microsatellite Instability and Other Genetic Data

	1p (n = 184)*		14q (n = 201)*		18q (n = 184)*		20q (n = 154)*				Microsatellite Status (n = 275)†	
	AI‡	P‖	AI‡	P‖	AI‡	P‖	Gain‡	P‖	Ampl‡	P‖	MSI§	P‖
Ploidy		NS		.006		NS		NS		.005		< .001
Diploid	29/39		20/55		44/23		25/23		12/23		32/77	
Aneuploid	55/61		58/68		85/32		28/24		42/24		4/162	
TP53		NS		.065		< .001		NS		.003		.001
Wild type	41/53		35/72		53/40		27/30		20/30		25/94	
Mutation	40/47		41/49		72/15		24/15		34/15		5/95	
Deletion at 17p13		NS		.081		< .001		NS		.002		< .001
Absent	21/29		15/36		21/24		15/19		7/19		23/49	
Present	49/55		52/67		84/23		31/21		37/21		4/136	

Abbreviations: AI, allelic imbalance; MSI, microsatellite instability; MSS, microsatellite stable; NS, not significant.

*Thirty-six tumors (1p), 19 tumors (14q), 36 tumors (18q), and 35 tumors (20q) were noninformative. Seven tumors showed deletions (20q), and 24 showed both gain and deletion (20q). These tumors were excluded from further statistical analyses.

†Data from Lothe et al,[4] Thorstensen et al,[67] and this study.

‡AI: number of tumors with allelic imbalance; number of tumors with retained heterozygosity; Gain: number of tumors with less than three times increases in copy number/number of tumors with retained heterozygosity; Ampl: number of tumors with three times or more increase in copy number (amplification)/number of tumors with retained heterozygosity.

§Number of tumors with MSI/number of tumors with MSS.

‖Comparison of different groups were tested with Pearson's χ^2 test or Fisher exact test. *P* values are two sided and are considered statistically significant when $P < .05$ and not significant when $P > .10$

Table 3. Frequencies and Types of Alteration at Microsatellite Loci Analyzed in 220 Primary Colorectal Carcinomas

Markers	Location	Genetic Changes (no.)				Retained Heterozygosity (no.)*	NonInformative (no.)
		Allolic Imbalance					
D1S199†	1p36			59		93	68
D1S2644†	1p36			54		77	89
D1S2647†	1p36			56		72	92
D14S276†	14q21q23			45		129	46
D14S48†	14q32			52		83	85
D14S265†	14q32			54		91	75
D18S535†	18q12			83		56	81
D18S51†	18q21			112		58	50
		Gain§	Ampl‖	Deletion	Deletion and gain		
D20S874‡	20q11	64	23	8	9	66	50
D20S855‡	20q12	58	16	5	2	87	52
D20S96‡	20q13	72	16	9	5	67	51
D20S891‡	20q13	54	29	4	5	79	49

Abbreviation: Ampl, amplification.

*Constitutional Heterozygous genotype is unchanged in the tumor.

†Analyzed by radioactive protocol.

‡Analyzed by fluorescent protocol.

§Gain: up to three times gain compared with the reference.

‖Ampl: three or more times gain compared with the reference.

was approved by the Norwegian Data Inspectorate for use and storage of patient's records.

The fresh frozen tumor tissues were mechanically minced in phosphate-buffered saline (pH 7.6), followed by nylon mesh filtration. The cells were fixed in 70% ethanol at 4°C. The degree of tumor cells versus normal cells in the tumor cell suspension was estimated as previously described,[68] and a mean of 84% tumor cells was determined, ranging from 62% to 97%. Blood and tumor samples were extracted with chloroform/phenol followed by ethanol precipitation using a model 340 ABI nucleic acid extractor (PE Biosystems, Foster City, CA).[69]

Allelotype Analysis

The selection of markers from 1p, 14q, 17p, and 18q was based on the previously identified smallest region of overlapping deletions determined within these chromosome arms.[26,27,34,70] Conversely, gains of the whole 20q have been reported from cytogenetic studies, and no amplicon has been identified.[26,63,71] Therefore, we examined markers distributed along this chromosome arm. The following 12 loci were analyzed: D1S199, D1S2644, D1S2647, D14S48, D14S265, D14S276, D18S51, D18S535, D20S96, D20S855, D20S874, and D20S891. The chromosomal map positions for these loci are given in Table 3. AI at 1p, 14q, 17p, and 18q loci reflected DNA sequence losses, but at the 20q loci, AI usually reflects variable amounts of copy number gains. Therefore, each 20q locus was amplified together with a control locus, known to reveal retained heterozygosity in the tumor in question. This enabled us to calculate the type and degree of imbalance observed at the 20q loci. The control loci used were D2S2194 (2p), D3S2748 (3q), D6S1575 (6p), D6S1589 (6q), D6S1596 (6q), and D12S1682 (12p). All the markers contained dinucleotide repeats, except D18S51 and D18S535, which contained tetra repeats, and were obtained from either Research Genetics (Huntsville, AL) or DNA Technology (Aarhus, Denmark). Primer sequences for polymerase chain reaction amplification, fragment size, and map positions were found in the Genome Database (http//:www.gdb.org).[72]

The protocols for the radioactive and for the fluorescent labeling of microsatellites, followed by gel and capillary electrophoresis, respectively, were described in detail by Skotheim et al.[73] Briefly, primers specific for each locus were used to amplify the repeat and flanking sequences in template DNA by polymerase chain reaction. In the radioactive protocol, the products were labeled with alpha-32-phoshporus deoxycytidine triphosphate (α-^{32}P-dCTP, Amersham Pharmacia Biotech Inc, Piscataway, NJ), separated by electrophoresis in 6% denaturing polyacrylamide gels, and visualized through autoradiography. AI was scored by visually comparing the tumor DNA pattern with the constitutional (blood) DNA pattern of each patient. A skewed intensity ratio between the two alleles in tumor DNA was considered as AI.

In the fluorescent protocol, the products were amplified with a fluorescent (6-carboxyfluorescein [6′-FAM], hexachlorinated 6′-FAM, or tetrachlorinated 6′-FAM; PE Biosystems) labeled primer. The fluorescent products were separated by capillary electrophoresis and analyzed by ABI PRISM 310 Genetic Analyzer (PE Biosystems) and further analyzed with Genescan and Genotyper software (PE Biosystems). The two alleles of each locus were assigned according to the two highest peaks, and degree of AI, Q^{LOH}, was determined. Q^{LOH} is calculated from the measured peak heights by dividing the allele ratio in tumor DNA by the allele ratio in the corresponding constitutional (normal) DNA. To distinguish between deletion and degree of gain at chromosome arm 20q, tumors with known AI were evaluated against control loci. Gain was defined as an increase of up to three times in copy number, whereas an increase of three times or more in copy number was defined as amplification. Degree of alteration on each locus was determined by the alteration ratio (AR). AR was defined as the ratio between marker (M) allele and reference (R) allele in tumor (T) and the corresponding constitutional (B; blood) DNA; AR for allele x; $x = 1$ or 2: $[M_{Bx}/(R_{B1}+R_{B2})/2]/[M_{Tx}/(R_{T1}+R_{T2})/2]$ (Fig 1). To compare visually scored AI with semiquantitative determined AI by fluorescent protocol, we have previously shown that a cutoff level of less than 0.75 (or inverse: > 1.33) should be used.[73] AI includes loss of heterozygosity, which indicates the complete absence of one allele. All of the scorings were performed independently by three of the authors (C.B.D., L.T., and R.A.L.) with few interobserver differences; these were resolved after joint re-evaluation. Samples with AI were confirmed by a second, independent analysis. Constitutional heterozygous genotypes remaining unchanged in the tumor are referred to throughout this article as retained heterozygosity.

MSI Analysis

In addition to the 12 microsatellites analyzed in this study, seven dinucleotide markers (D1S216, D5S404, D8S255, D10S197, D11S904, D13S175, and D17S787) and two mononucleotide markers (BAT25 and BAT26) have previously been analyzed for MSI in this series of CRCs.[4,67] The following criteria were used to determine the MSI status: tumor samples showing MSI in $\geq$ 30% of the analyzed loci or having mutation in both BAT25 and BAT26 were classified as microsatellite high, whereas samples with MSI in at least one locus and in less than 30% of the loci ($n = 21$) analyzed were considered as microsatellite low. Samples with no MSI were classified as microsatellite stable (MSS).[74] In total, the MSI status has been determined for 275 carcinomas from 275 patients (present study).[4,67] Similarity between microsatellite low and MSS tumors have previously been

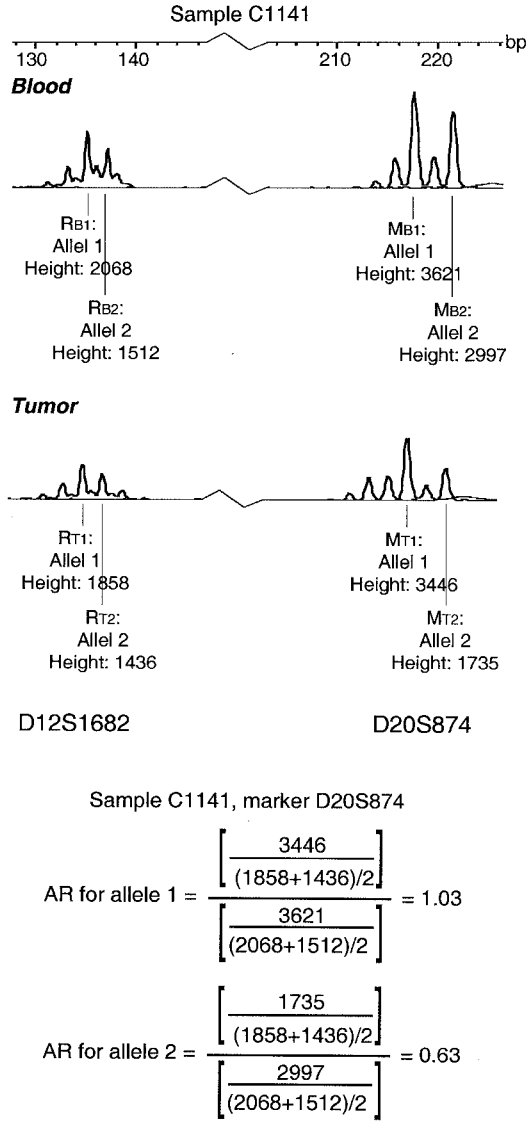

Fig 1. Sample C1141 had a decrease in copy number for allele 2 at locus D20S874.

shown, and therefore, these groups are dealt with as MSS throughout the text.[4,74] Microsatellite high is referred to as MSI.

TP53 Mutations and 17p13 Losses

Mutation analyses of *TP53* in exon 5–8 have previously been reported.[44] All samples with aberrant migrating bands by constant denaturant gel electrophoresis were submitted to direct sequencing. Among the tumors included in studies by Børresen-Dale et al[44] and the present study, 86 (44%) of 196 tumors had *TP53* mutations.

By Southern blot analyses, AI has previously been found at pBHP53 and D17S30 in 231 CRCs, including 216 tumors from present series.[36] Both loci are located at chromosome band 17p13. Deletions at one or both markers were found in 124 (68%) of 182 informative tumors included in the present study.

DNA Ploidy

The measurement of total DNA content, the DNA ploidy pattern, has been performed on this series of CRCs,[36] and 84 (38%) and 136 (62%) of the present samples were classified as DNA diploid and DNA aneuploid, respectively.

Statistical Analysis

Comparisons of different groups were tested with Pearson's χ^2 test or Fisher's exact test. Cause-specific survival analysis (death by CRC) was performed by Cox regression and the Kaplan-Meier method, and the differences in survival were assessed using the log-rank test. The following variables were included in the Cox regression analysis: age, sex, Dukes' stage, tumor site, histologic grade, DNA ploidy pattern, MSI status, and genotype changes at chromosome arms 1p, 14q, 17p, 18q, and 20q. Survival analysis of MSI status, 17p deletions, and *TP53* mutations were performed for all patients included in the present and our previous studies.[4,36,44,67] Only *P* values lower than .05 were regarded as statistically significant, and all of the statistical analyses were performed with the use of SPSS software (SPSS, Chicago, IL).

RESULTS

Analysis of AI and MSI

Among the informative cases (defined as constitutional heterozygosity in the corresponding blood DNA) 84 (46%) of 184 carcinomas showed AI at one or more loci on chromosome arm 1p, 78 (39%) of 201 tumors showed AI on 14q, 129 (70%) of 184 tumors showed AI on 18q, and 138 (75%) of 185 tumors showed AI on 20q. The alterations on chromosome arm 20q included the following: 53 (39%) tumors with gain, 54 (39%) tumors with amplification, seven (5%) tumors with deletion (Fig 1), and 24 (17%) tumors with both gain and deletion. Tumors with deletion or both gain/amplification and deletion were excluded from the association analyses. The frequency and type of alteration at each locus are summarized in Table 3. All tumor DNAs were informative in at least five (median, 20 loci; range, five to 21 loci) of the microsatellite loci analyzed. Thirty-six (13%) of 275 informative tumors were classified as MSI. Exclusion of the few samples showing both MSI and AI did not have any statistical effects on the association or survival analyses; therefore, these tumors were included in the analyses.

Genetic and Clinicopathologic Associations

Associations between the present results and clinicopathologic data, as well as additional genetic data, are summarized in Tables 1 and 2. AI at 14q loci was more frequent in aneuploid tumors than in diploid tumors ($P = .006$). Tumors with AI at chromosome arm 18q contained *TP53* mutations and losses of chromosome band 17p13 ($P < .001$ and $P < .001$, respectively). Amplification of 20q was associated with the presence of *TP53* mutation ($P = .003$), deletion of chromosome band 17p13 ($P = .002$), and aneuploidy ($P = .005$). MSI tumors were associated with right-sided location ($P < .001$), poor differentiation ($P < .001$), diploidy ($P < .001$), absence of *TP53* mutation ($P = .001$), and retained heterozygosity at 17p13 loci ($P < .001$).

Survival Associations

A significant association between Dukes' stages and survival was confirmed in the present series ($P < .001$, Table 4). Patients with tumors harboring losses of chromosome arms 17p or 18q showed a shorter survival compared with patients without these alterations ($P = .021$ and $P = .008$, respectively, Fig 2A and 2B). Evaluation of the prognostic impact of 17p and 18q losses in relation to the different Dukes' classes showed a significant association between either of these changes and survival for Dukes' stage B patients ($P = .025$, $P = .010$, Fig 2C and 2D).

Table 4. Hazard Ratio and 95% Confidence Intervals for Tumor Genotype and Dukes' Stages Derived from Cox Regression Analyzes

Parameters	Clinical Groupings	Unadjusted Cox Regression			Adjusted Cox Regression		
		HR	95% CI	*P*	HR	95% CI	*P*
Changes at 17p	AI versus no AI	1.97	1.14 to 3.39	.015	2.76	1.31 to 5.83	.008
Changes at 18q	AI versus no AI	2.70	1.33 to 5.48	.006	—	—	—
Changes at 20q	Retained heterozygosity*	1	—	.049	1	—	.012
	Gain†	0.46	0.22 to 0.98	.043	0.32	0.13 to 0.75	.009
	Amplification†	1.16	0.63 to 2.12	.639	1.14	0.60 to 2.16	.697
Dukes' Stage	A versus B versus C versus D	3.41	2.55 to 4.56	< .001	4.47	2.80 to 7.15	< .001
TP53	‡	1.84	1.11 to 3.04	.018	—	—	—

Abbreviations: HR, hazard ratio; CI, confidence interval; AI, allelic imbalance.

*Reference for gain and amplification.

†Gain: up to three times increase in copy number; amplification: three or more times increase in copy number.

‡Mutation affecting L3-zinc finger domain versus other mutations or absence of mutation in *TP53*.

Patients with tumors showing losses of both chromosome arms (17p and 18q) revealed an even poorer disease outcome than those with either 17p or 18q loss or those without losses ($P = .008$, Fig 2E). The same association was also evident within the Dukes' stage B group ($P = .041$, Fig 2F). The impact of alteration of 17p was also significant in regression analysis adjusted for other covariates (Table 4).

Patients with tumors containing gain of 20q had a better clinical outcome than patients with retained heterozygosity and patients with amplification of this chromosome arm ($P = .009$ and $P = .037$, respectively; Fig 3A). This relation held also for patients in the Dukes' stage C group ($P = .018$ and $P = .030$, respectively; Fig 3B). Patients with tumors harboring MSI showed a trend toward a better survival compared with patients with MSS tumors, although it was not statistically significant ($P = .071$, Fig 3C).

A significant relationship between mutations affecting the L3 zinc-binding domain of *TP53* and lower survival rate was found in the whole cohort as well as for patients in the Dukes' stage B group ($P = .016$ and $P = .032$, respectively; Fig 4A and 4B). This trend was also seen for Dukes' stage C patients but did not reach the chosen statistical significance level ($P = .071$). No associations were found between alterations on 1p, 14q, or *TP53* mutation (independent of localization and type) and survival of the patients ($P = .381$, $P = .207$, and $P = .184$, respectively).

DISCUSSION

Previous studies[26,27,34,63,71] have identified frequent deletions as well as the smallest regions of overlapping alterations within chromosome arms 1p, 14q, 17p, and 18q in CRC. Our series of CRCs was comparable with other series in that the frequencies of changes at 1p, 14q, 17p, and 18q (46%, 39%, 66%, and 70%, respectively) were in agreement with previous reports.[22,24,31,36,75-77] To our knowledge, AI at 20q has not been reported in large CRC series, but the high frequency of 20q changes is consistent with results from comparative genomic hybridization (CGH) studies.[62,66,71,78-83] If alterations were found, the different loci at the individual chromosome arms usually showed the same change, suggesting that the alterations most often reflected relatively large chromosomal changes in the tumor in question.

It is well known that tumors located in the right versus the left part of the large bowel have different spectra of genetic alterations and have characteristic clinicopathologic profiles.[3-6,53,84,85] The present report confirmed that MSI tumors were mainly right-sided, poorly differentiated, diploid, and rarely exhibited *TP53* mutations. Furthermore, chromosome instable tumors with AI at 18q also contained *TP53* mutations and 17p deletions. We found striking associations between aneuploidy and AI at 14q and amplification of 20q that were not previously reported. Both of these chromosomal alterations were frequently seen in tumors with *TP53* mutation or 17p deletion, although statistical significance was only reached for tumors with 20q amplification. These findings suggest that, in addition to changes of 17p and 18q, deletion of 14q and amplification of 20q are part of the chromosomal instability phenotype that is characteristic for a major subgroup of CRCs.[8] We have previously found an association between deletions of 1p sequences and adenomas located in the distal colon and rectum,[25] and this has been reported by others in carcinomas.[86] However, we could not confirm this in the present series of carcinomas.

Various studies have aimed to find associations between genetic changes in tumor and the disease outcome for the patient. However, many of these studies were based on a small number of patients from nonconsecutive series.[58,87] The present consecutive series included 220 white patients from the same geographic region who were treated with surgery and followed for 10 years. In 1989, Kern et al[53] presented the first study that showed an association between allelic losses of 17p and 18q and poor survival. Several additional studies have confirmed this association[12,16,42-52]; however, others have not.[9,45,52,54-56] These differences may be explained by variation in series size, selection of patients included, and variation in follow-up time. The present study demonstrated an impact of 17p and 18q deletions on survival for CRC patients who had undergone curative surgery. In addition, the Dukes' stage B patients could be subdivided into two groups with significantly different prognosis dependent on the status of the 17p and 18q tumor markers. Finally, the combined status, in which both chromosome arms were affected, showed even a worse disease outcome.

Several potential tumor suppressor genes have been mapped to chromosome band 18q21, including *DCC*,[39] *SMAD2*,[40] and *SMAD4*.[41] *DCC* has rarely been found mutated in colorectal tumors, whereas *SMAD2* and *SMAD4* are found mutated in 11% and 30%, respectively.[88,89] The tumor suppressor gene *TP53* is

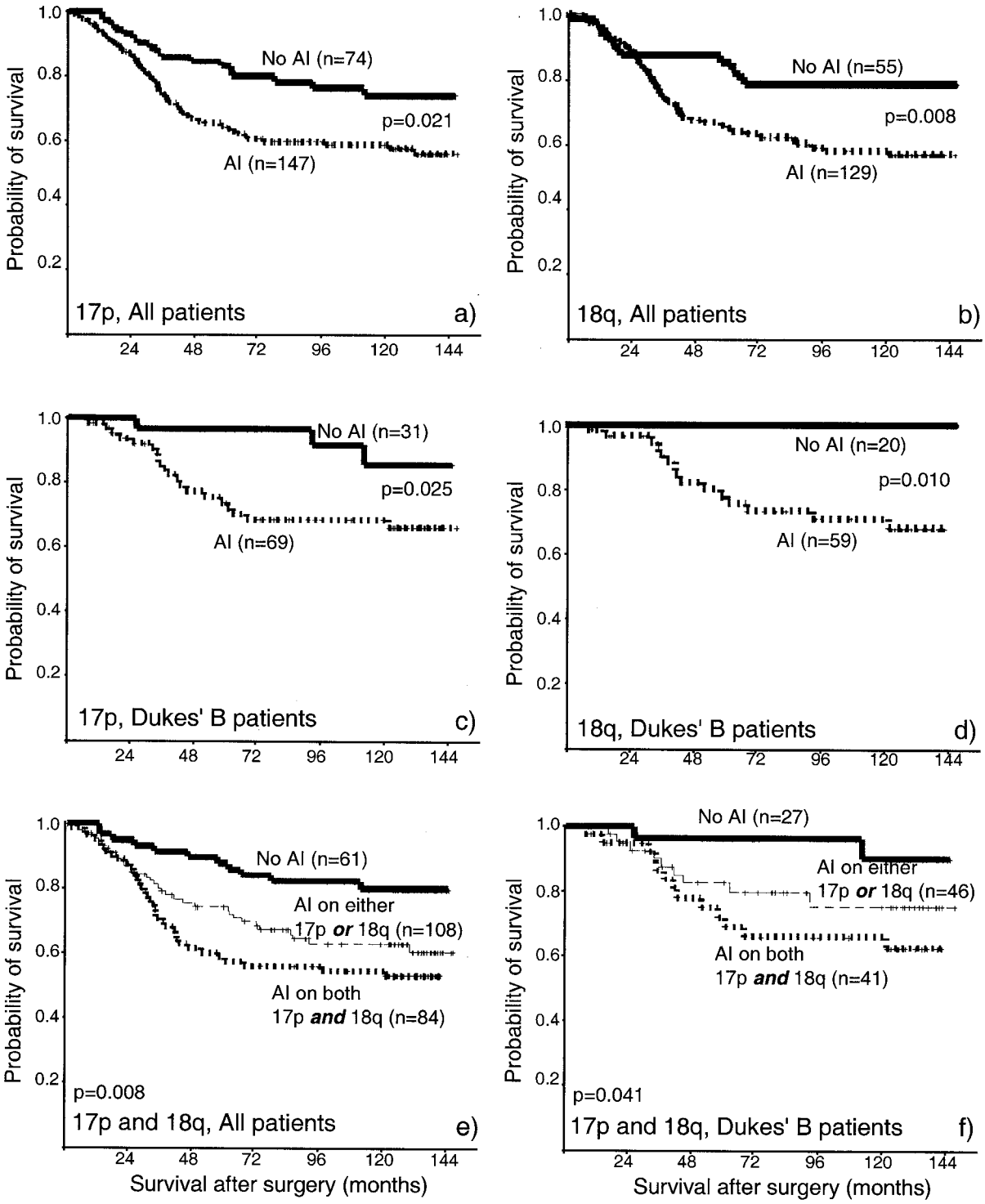

Fig 2. Changes at 17p (A, C) and 18q (B, D) were associated with poor clinical outcome in colorectal cancer patients. (E, F) Patients with alterations on both chromosome arms had a lower survival rate than patients without allelic imbalance (AI) on one or both of these arms.

the target gene at 17p13, and conflicting results exist with regard to whether *TP53* mutations predict poor survival.[57,58] Our initial report of the same series with 5 years of observation time showed that only mutations affecting the L3 zinc-binding domain were associated with poor survival.[44] The present report confirmed this ($P = .016$) in the same cohort after 10 years of follow-up. Finally, subclassification of the patients into Dukes' stages suggested that prognosis evaluation of both the B and C group could benefit from knowledge of the *TP53* mutation status.

For yet unknown reasons, gain (increase of up to three times in copy number) of 20q is associated with improved prognosis compared with amplification or no change at this chromosome arm. This was independent of Dukes' classification and separated the Dukes' stage C patients into two significantly

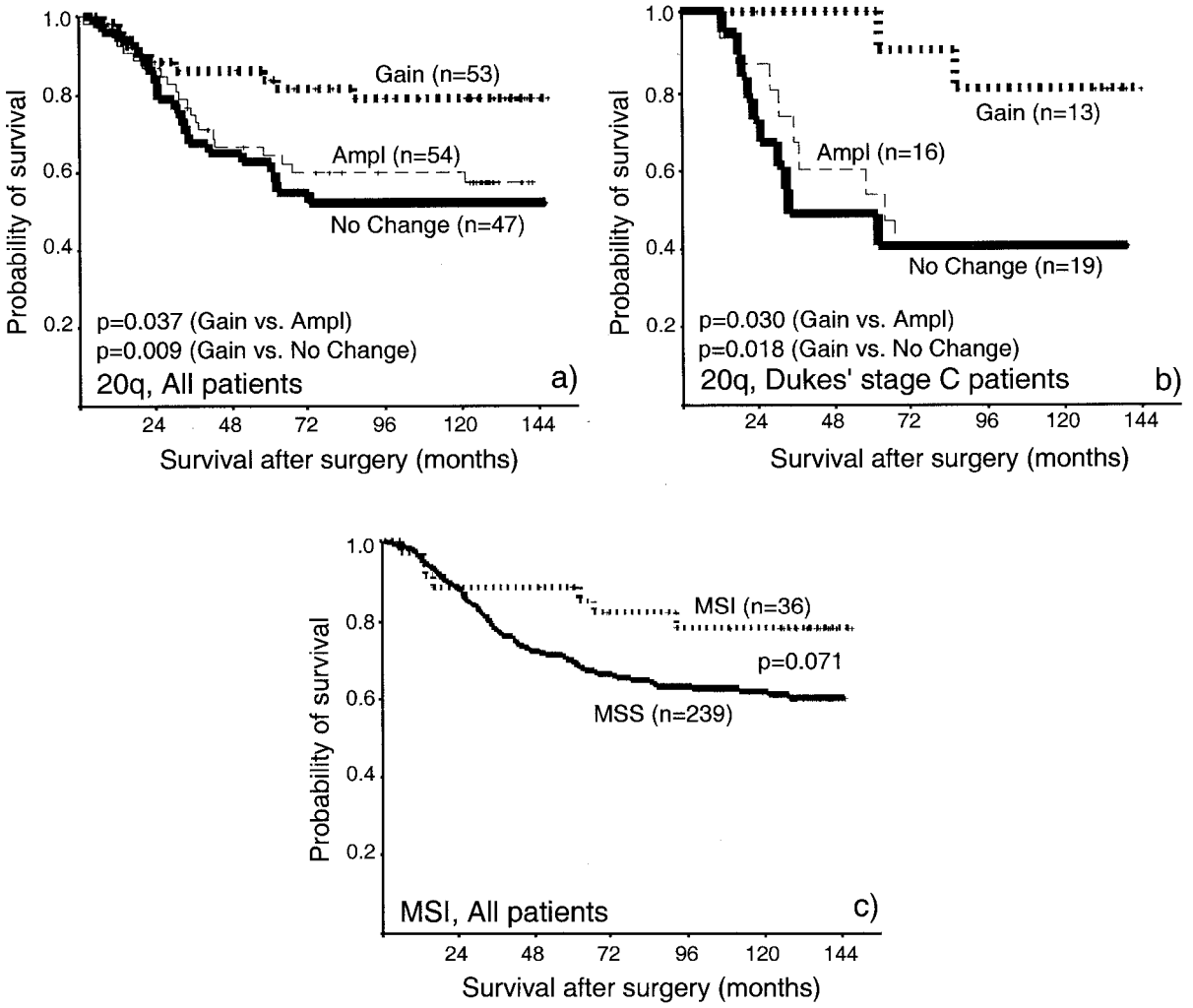

Fig 3. (A and B) Patients with gain of 20q had better outcome compared with patients with retained 20q or those with amplification of 20q. The same was seen in patients in Dukes' C stage. (C) MSI was seemingly also a beneficial tumor marker for survival as end point.

different groups. The present study is the first that reports a clinical benefit for patients with tumors containing a low copy number increase of 20q. One previous report has studied 20q changes in association with clinical outcome in patients with CRC, and they reported that gain of 20q resulted in shortened patient survival.[71] These different results may be explained by the different number of patients (220 in this study *v* 67 in the previous) included in the studies, sampling differences (consecutive *v* not), different methods used (microsatellite analysis *v* CGH), and different interpretation of the results (the former study did not distinguish between low and high copy number gain).

In the present study, neither deletions of 1p nor of 14q sequences exhibit statistically significant associations to survival. To our knowledge, three reports have studied the relationship between alterations of 1p and survival.[42,71,90] Two of these studies[42,71] reported that loss of 1p was associated with shorter survival time. The third study[90] did not find any relationship to survival. They found, however, prognostic impact of selected markers on 1p. All previous reports included a smaller number of patients (n = 47, n = 67, and n = 116) than the present study, and different methods have been used (restriction fragment length polymorphism, CGH, cytogenetic banding, and microsatellite analysis). De Angelis et al[71] reported a relationship between deletion of 14q and survival, which cannot be confirmed in the present study. These different results may be explained by the same arguments as suggested for the 1p data above. Although we could not find that AI at 1p or 14q was associated with survival, the fact that these chromosome regions are frequently altered in the primary carcinomas as well as in their metastases[23,26,63] suggests that they are important contributors to tumor progression but not likely contributors to aggressiveness.

MSI in CRC was first described in 1993,[3-6] and several studies have examined this phenomenon and clinical outcome. The conclusions of the different studies are conflicting; some find MSI to be associated with prolonged survival time for CRC patients,[4,5,9-15,17,18] whereas others do not.[16,19-21] In the present study, the patients with MSI seemed to have a better disease outcome than patients without MSI tumors (P = .071). This trend is in agreement with our initial report on the same cohort,[4] in which we found significant prolonged survival for patients with microsatellite unstable tumors (P = .05). The present study indicated that changes at 17p, 18q, and

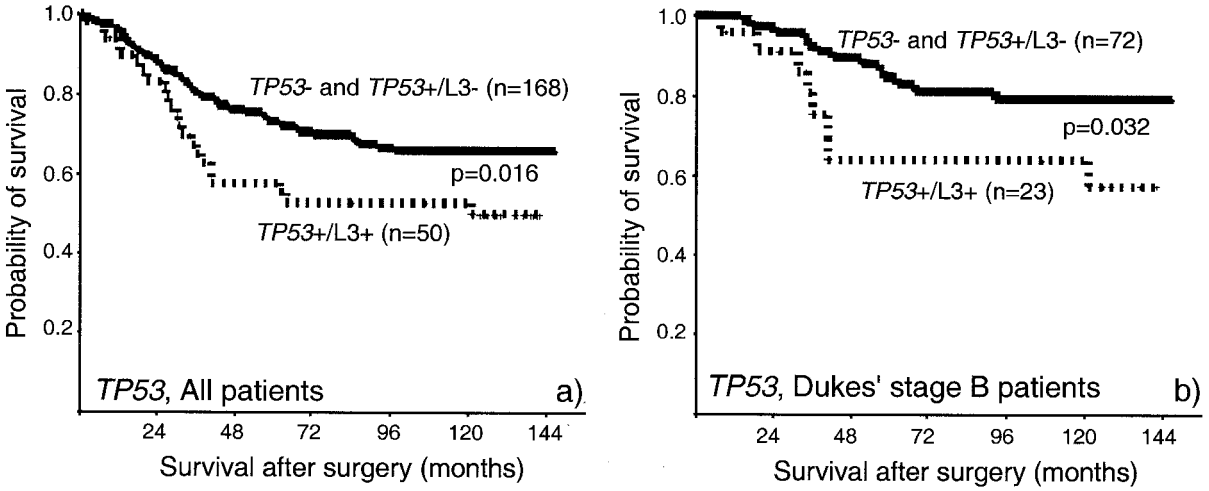

Fig 4. (A) Patients with mutation in L3 zinc-binding domain in *TP53* had significantly poorer outcome compared with patients without mutation in L3 zinc-binding domain. (B) The same was seen in patients in the Dukes' B group.

20q, and *TP53* status add information in the subclassification of Dukes' stages B and C patients and, thus, may have impact on the choice of treatment.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of Merete Hektoen.

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