Prognostic Significance and Molecular Associations of 18q Loss of Heterozygosity: A Cohort Study of Microsatellite Stable Colorectal Cancers

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A B S T R A C T

Purpose

Loss of heterozygosity (LOH) at chromosome 18q frequently occurs late during colon cancer development and is inversely associated with microsatellite instability (MSI). 18q LOH has been reported to predict shorter survival in patients with colorectal cancer, whereas MSI-high status has been associated with superior prognosis. However, it is unclear whether 18q LOH in colorectal cancer has any prognostic implication independent of MSI status and other potential predictors of clinical outcome.

Patients and Methods

Among 555 non-MSI-high colorectal cancers (stage I to IV) in two independent prospective cohort studies, we examined 18q LOH in relation to other molecular events and patient survival. Cox proportional hazard models computed hazard ratio of death, adjusted for clinical and tumoral characteristics, including *KRAS*, *BRAF*, *PIK3CA*, β -catenin, p53, CpG island methylator phenotype, LINE-1 methylation, and John Cunningham (JC) virus T antigen.

Results

In multivariate logistic regression, 18q LOH was independently associated with JC virus T antigen (odds ratio [OR] = 1.93; $P = .0077$), body mass index ≥ 30 kg/m² (obesity; OR = 2.01; $P = .014$), high tumor grade (OR = 0.40; $P = .018$), *KRAS* mutation (OR = 0.66; $P = .40$), and LINE-1 hypomethylation (for a 30% decrease; OR = 1.92; $P = .045$). Five-year colorectal cancer-specific survival was 75% among patients with 18q LOH-positive tumors and 74% among those with 18q LOH-negative tumors (log-rank $P = .80$). Five-year overall survival was 70% among patients with 18q LOH-positive tumors and 68% among those with 18q LOH-negative tumors (log-rank $P = .54$). Multivariate analysis did not show prognostic significance of 18q LOH.

Conclusion

In our large prospective study of patients with non-MSI-high colorectal cancer, 18q LOH or allelic imbalance was not associated with patient survival.

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INTRODUCTION

Colorectal cancers develop through a multistep carcinogenic process in which genetic and epigenetic events sequentially accumulate.¹ Allelic loss at chromosome 18q, which can be assessed by loss of heterozygosity (LOH) analysis, frequently occurs late in the carcinogenic process. There are many candidate tumor suppressor genes in 18q, including *DCC*, *SMAD4* (DPC4), *SMAD2*, and *CABLES1*.²⁻⁴ 18q LOH has been inversely associated with microsatellite instability (MSI),⁵ which is an important molecular classifier in colorectal cancer.^{6,7}

The presence of tumoral 18q LOH has been associated with an inferior survival in colorectal can-

cer in some studies,⁸⁻¹¹ though not all.¹²⁻¹⁵ Many of the previous studies assessing the prognostic significance of 18q LOH^{8,9,12,16} did not consider the potential confounding effect of MSI, which is typically associated with improved patient survival.¹⁷ Moreover, a meta-analysis of research articles on 18q LOH and clinical outcome in colorectal cancers showed strong evidence for publication bias,¹⁸ in that small studies with null results had a higher likelihood of being unpublished, as compared with small studies with "significant" results. Although three large-scale studies ($N > 300$) have failed to show independent prognostic significance of 18q LOH in colorectal cancer,¹³⁻¹⁵ one study with 279 informative cases of colorectal cancer did find that

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18q LOH was independently associated with a significant reduction in patient survival.⁵ Additional well-conducted large-scale studies are necessary to draw definitive conclusions.

In this study using more than 500 stage I to IV colorectal cancers identified in two independent, prospective cohort studies, we examined the prognostic significance and molecular associations of 18q LOH in non-MSI-high tumors. Because we concurrently assessed related molecular variables such as the CpG island methylator phenotype (CIMP), LINE-1 hypomethylation, p53, and *BRAF* and *KRAS* mutations, we could evaluate the effect of 18q LOH independent of these potential confounders.

PATIENTS AND METHODS

Study Population

We used the databases of two independent prospective cohort studies: the Nurses' Health Study (N = 121,701 women observed since 1976)^{19,20} and the Health Professionals Follow-Up Study (N = 51,529 men observed since 1986).²⁰ Every 2 years, participants have been sent follow-up questionnaires to identify newly diagnosed cancer. We collected paraffin-embedded tissue blocks from hospitals where patients underwent tumor resections.²⁰ We excluded cases preoperatively treated with radiation and/or chemotherapy. Tissue sections from all colorectal cancer cases were reviewed by the pathologist (S.O.). Tumor grade was categorized as high (poor or undifferentiated) versus low (moderate or well differentiation), defined as $\leq 50\%$ versus more than 50% glandular area, respectively. We excluded 62 non-MSI-high tumors because of lack of informative 18q LOH results. On the basis of availability of tissue specimens and informative results of 18q LOH, we included a total of 555 non-MSI-high colorectal cancers diagnosed up to 2002. Written informed consent was obtained from all study subjects. This study was approved by the human subjects committees at Brigham and Women's Hospital and the Harvard School of Public Health.

Measurement of Mortality

Patients were observed until death or June 30, 2006, whichever came first. Ascertainment of deaths included reporting by the family or postal authorities. In addition, the names of persistent nonresponders were searched in the National Death Index. The cause of death was assigned by physicians blinded to information on lifestyle exposures and tumoral molecular changes.

DNA Extraction and Pyrosequencing of *KRAS*, *BRAF*, and *PIK3CA*

DNA from paraffin-embedded tissue was extracted, and pyrosequencing targeted for *KRAS* codons 12 and 13,²¹ *BRAF* codon 600,²² and *PIK3CA* exons 9 and 20 were performed.²³

MSI and 18q LOH Analysis

MSI status was determined using 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487).²⁴ MSI high was defined as the presence of instability in $\geq 30\%$ of the markers, MSI low was defined as the presence of instability in 10% to 29% of the markers, and microsatellite stability was defined as no unstable marker. In this study, we examined non-MSI-high tumors to assess the clinical and molecular correlates with 18q LOH, because MSI high, if present, often made interpretation of 18q microsatellite markers difficult or impossible, and because MSI-high tumors rarely exhibited 18q LOH.

For LOH analysis, we duplicated polymerase chain reaction (PCR) reaction and electrophoresis for 18q markers (D18S55 at 18q22.1, D18S56 at 18q12.2-3, D18S67 at 18q12.2-3, and D18S487 at 18q21.1) to exclude allele dropouts of one of two alleles.²⁴ LOH at each locus (D18S55, D18S56, D18S67, or D18S487) was defined as $\geq 40\%$ reduction of one of two allele peak heights in tumor DNA relative to normal DNA. 18q LOH positivity was defined as the presence of LOH in any of the 18q markers. 18q LOH negativity was strictly defined as the presence of at least two informative markers and the absence of LOH.

Real-Time PCR for CpG Island Methylation and Pyrosequencing to Measure LINE-1 Methylation

Sodium bisulfite treatment on tumor DNA and subsequent real-time PCR (MethylLight) assays were validated and performed.²⁵ We quantified promoter methylation in eight CIMP-specific genes (*CACNA1G*, *CDKN2A*, *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3*, and *SOC1*).²⁶⁻²⁸ CIMP high was defined as six or more of eight methylated promoters using the eight-marker CIMP panel, and CIMP low/0 was defined as 0 to five methylated promoters, according to the previously established criteria.²⁷ To accurately quantify relatively high LINE-1 methylation levels, we used pyrosequencing as previously described.²⁹

Immunohistochemistry for p53, β -catenin, and John Cunningham Virus T Antigen

Tissue microarrays were constructed,^{20,30} and immunohistochemistry was performed as previously described for John Cunningham virus T antigen (JCVT),³¹ p53,³² and β -catenin.³³ All immunohistochemically stained slides were interpreted by a pathologist (p53 by S.O., β -catenin and JCVT by K.N.) unaware of other data. A random sample of 118 to 402 tumors were re-examined for each marker by a second observer (p53 by K.N., β -catenin by S.O., and JCVT by Y.B.) unaware of other data, and the concordance between the two observers was 87% for p53 ($\kappa = 0.75$, $n = 118$), 83% for β -catenin ($\kappa = 0.65$, $n = 402$), and 87% for JCVT ($\kappa = 0.74$, $n = 147$), indicating substantial agreement.

Statistical Analysis

We used SAS program (version 9.1, SAS Institute, Cary, NC). All *P* values were two-sided, and statistical significance was set at $P < .05$. Nonetheless, *P* values were conservatively interpreted when conducting multiple hypothesis testing. For categorical data, the χ^2 test (or Fisher's exact test if appropriate) was performed. The κ coefficient was calculated to assess an agreement between the two observers in immunohistochemical analyses. We also examined the possibility of a nonlinear relation of body mass index (BMI) or LINE-1 methylation with 18q LOH, nonparametrically with restricted cubic splines.³⁴

To assess independent relations of multiple variables with 18q LOH, a multivariate logistic regression analysis was performed. Odds ratio (OR) was adjusted for sex, age (continuous), BMI (< 30 v ≥ 30 kg/m²), family history of colorectal cancer (present v absent), tumor location (proximal v distal), tumor stage (I to II v III to IV), tumor grade (low v high), CIMP status (high v low/0), MSI status (low v microsatellite stability), LINE-1 methylation (continuous), *BRAF*, *KRAS*, *PIK3CA*, p53, β -catenin, and JCVT. For BMI, tumor grade, and JCVT, we assigned separate indicator ("missing") variables to those cases with missing data. For cases missing LINE-1 data, we assigned the mean LINE-1 methylation level. For cases with missing information in any of the other variables (tumor location, tumor stage, CIMP, *BRAF*, *KRAS*, *PIK3CA*, and p53), we included those cases in a majority category to not use more than 1 *df* for each variable in a multivariate model. We confirmed that excluding cases with missing information in any of the variables did not substantially alter results (data not shown).

For survival analysis, the Kaplan-Meier method and log-rank test were used. For analyses of colorectal cancer-specific mortality, deaths not resulting from colorectal cancer were censored. To assess independent effect of 18q LOH on mortality, we constructed a multivariate, stage-matched (stratified) Cox proportional hazard model to compute a hazard ratio (HR). Tumor stage (I, IIA, IIB, IIIA, IIIB, IIIC, IV, unknown) was used as a matching (stratifying) variable, and other adjustment was performed as in the multivariate logistic regression. The proportionality of hazards assumption was satisfied by evaluating time-dependent variables, which were the cross-product of the 18q LOH variable and survival time ($P = .31$ for colon cancer-specific mortality; $P = .077$ for overall mortality). An interaction was assessed by including the cross-product of the 18q LOH variable and another variable of interest in a multivariate Cox model, and the Wald test was performed.

18q LOH in Colorectal Cancer

Table 1. Clinical and Molecular Features of Non-MSI-High Colorectal Cancer According to 18q LOH Status

Clinical or Molecular Feature	Total		18q LOH Negative		18q LOH Positive		P
	No.	%	No.	%	No.	%	
Total No. of patients	555		193		362		
Sex							.36
Male, HPFS	244	44	90	47	154	43	
Female, NHS	311	56	103	53	208	57	
Age, years							.27
Mean	66.8		67.3		66.5		
SD	8.3		8.4		8.2		
Body mass index, kg/m ²							.018
< 30	424	82	154	88	270	80	
≥ 30	90	18	21	12	69	20	
Family history of colorectal cancer in first-degree relative(s)							.55
No	428	77	146	76	282	78	
Yes	127	23	47	24	80	22	
Year of diagnosis							.42
Before 1995	223	40	82	42	141	39	
1995 to 2002	332	60	111	58	221	61	
Tumor location							.025
Proximal, cecum to transverse	217	40	89	48	128	36	
Distal colon, splenic flexure to sigmoid	178	33	50	27	128	36	
Rectum	144	27	48	26	96	27	
AJCC tumor stage							.26
I	131	24	43	22	88	24	
IIA	122	22	42	22	80	22	
IIB	13	2.3	7	3.6	6	1.7	
IIIA	24	4.3	12	6.2	12	3.3	
IIIB	75	14	19	9.8	56	15	
IIIC	64	12	23	12	41	11	
IV	84	15	29	15	55	15	
Unknown	42	7.6	18	9.3	24	6.6	
Tumor grade							.0060
Low	506	93	167	89	339	95	
High	36	6.6	20	11	16	4.5	
MSI status							.80
MSS	495	89	173	90	322	89	
MSI low	60	11	20	10	40	11	
CIMP, No. of methylated CIMP markers							.095
CIMP 0	272	50	82	44	190	54	
CIMP low (1-5)	233	43	90	48	143	41	
CIMP high (6-8)	34	6.3	14	7.5	20	5.7	
LINE-1 methylation, %							.040
Mean	60.7		61.9		60.1		
SD	9.3		9.5		9.2		
BRAF mutation							.45
Negative	497	91	170	90	327	92	
Positive	48	8.8	19	10	29	8.2	
KRAS mutation							.015
Negative	330	60	101	53	229	63	
Positive	224	40	91	47	133	37	
PIK3CA mutation							.11
Negative	438	85	145	82	293	87	
Positive	75	15	32	18	43	13	
p53 expression							.071
Negative	292	53	110	59	182	51	
Positive	254	47	77	41	177	49	
β-catenin activation*							.30
Inactive (score 0-1)	207	43	75	46	132	41	
Active (score 2-5)	277	57	88	54	189	59	
JC virus T antigen							.0004
Negative	285	60	120	71	165	54	
Positive	190	40	50	29	140	46	

NOTE. Table indicates the proportion of tumors with a specific clinical, pathologic, or molecular feature in all patients or patients with 18q LOH-negative tumors or 18q LOH-positive tumors.

Abbreviations: MSI, microsatellite instability; LOH, loss of heterozygosity; HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study; SD, standard deviation; AJCC, American Joint Committee on Cancer; MSS, microsatellite stable; CIMP, CpG island methylator phenotype; JC, John Cunningham.

*β-catenin was assessed by immunohistochemistry and activation score was calculated as previously described.³³

Fig 1. 18q loss of heterozygosity (LOH) in colorectal cancer. (A) Smoothing spline plot of odds ratio (OR) for 18q LOH in non-microsatellite instability (MSI)-high colorectal cancer according to body mass index (BMI) (left panel) and LINE-1 methylation level (right panel). Patients with low BMI or with high-level LINE-1 methylation were used as a reference group; 95% CIs are indicated by blue dashed lines. (B) Kaplan-Meier curves for colorectal cancer-specific survival (top panel) and overall survival (bottom panel) according to 18q LOH status in non-MSI-high colorectal cancer.

Table 2. Multivariate Analysis of the Relations With 18q LOH in 555 Non-MSI-High Colorectal Cancers

Variable Independently Associated With 18q LOH	Multivariate OR	95% CI	P
JC virus T antigen	1.93	1.19 to 3.13	.0077
BMI ≥ 30 kg/m ² v < 30 kg/m ²	2.01	1.15 to 3.52	.014
High tumor grade v low grade	0.40	0.19 to 0.86	.018
KRAS mutation	0.66	0.44 to 0.98	.040
LINE-1 hypomethylation, 30% decrease as a unit	1.92	1.02 to 3.62	.045

NOTE. The multivariate logistic regression model included age, sex, family history of colorectal cancer, tumor location, stage, MSI (low v microsatellite stability), CIMP, BRAF, PIK3CA, p53, β -catenin, and the variables listed in the table.

Abbreviations: LOH, loss of heterozygosity; MSI, microsatellite instability; OR, odds ratio; JC, John Cunningham; BMI, body mass index.

RESULTS

18q LOH in Non-MSI-High Colorectal Cancer

Using 555 non-MSI-high colorectal cancers with informative 18q LOH data, we detected LOH in at least one 18q marker in 362 tumors (65%; Table 1). Tumors with 18q LOH were associated with obesity (BMI ≥ 30 kg/m²; $P = .018$), distal colon ($P = .025$), low tumor grade ($P = .0060$), low-level LINE-1 methylation ($P = .040$), wild-type KRAS ($P = .015$), and JCVT ($P = .0004$).

We examined the possibility of nonlinear relations of BMI and LINE-1 methylation with 18q LOH, nonparametrically with restricted cubic splines³⁴ (Fig 1A). This flexible method allowed us to examine the relation to 18q LOH without any categorization of BMI or LINE-1 methylation level. It was evident that odds for 18q LOH increased as BMI increased or as LINE-1 methylation level decreased.

Multivariate Analysis for Relations With 18q LOH

In multivariate logistic regression analysis (Table 2), 18q LOH was independently associated with JCVT (OR = 1.93; 95% CI, 1.19 to 3.13; $P = .0077$), BMI ≥ 30 kg/m² (OR = 2.01; 95% CI, 1.15 to 3.52; $P = .014$), and LINE-1 hypomethylation (for a 30% decrease; OR = 1.92; 95% CI, 1.02 to 3.62; $P = .045$), and inversely with high tumor grade (OR = 0.40; 95% CI, 0.19 to 0.86; $P = .018$) and KRAS mutation (OR = 0.66; 95% CI, 0.44 to 0.98; $P = .040$). Nonetheless, considering multiple hypothesis testing, any of these associations could be a chance event.

18q LOH and Patient Survival in Non-MSI-High Colorectal Cancer

Using our cohort database, we previously demonstrated that molecular features in colon cancer, such as BRAF mutation, fatty acid synthase expression, PIK3CA mutation, and LINE-1 hypomethylation, were significantly associated with prognosis.³⁵⁻³⁸ We assessed the influence of 18q LOH status on patient survival. During follow-up of 532 patients who were eligible for survival analysis, there were a total of 239 deaths, including 155 colorectal cancer-specific deaths. Five-year colorectal cancer-specific survival was 75% among patients with 18q LOH-positive tumors and 74% among those with 18q LOH-negative tumors (log-rank $P = .80$; Fig 1B). Five-year overall survival was 70% among patients with 18q LOH-positive tumors and 68% among those with 18q LOH-negative tumors (log-rank $P = .54$).

In univariate Cox regression analysis, compared with patients with 18q LOH-negative tumors, patients with 18q LOH-positive tumors experienced similar colorectal cancer-specific mortality (HR = 0.96; 95% CI, 0.69 to 1.33; Table 3). We examined the prognostic effect of 18q LOH after adjusting for potential predictors of patient survival, including age, sex, BMI, family history of colorectal cancer, year of diagnosis, tumor location, stage, grade, CIMP, LINE-1 hypomethylation, p53, β -catenin, KRAS, BRAF, and PIK3CA. Compared with patients with 18q LOH-negative tumors, patients with 18q LOH-positive tumors experienced similar colorectal cancer-specific mortality (multivariate HR = 1.18; 95% CI, 0.81 to 1.71). When overall mortality was used as an end point, results were similar (Table 3).

In addition, we examined whether LOH status at each of the four microsatellite loci could predict patient survival. LOH at any of the 18q loci was not significantly associated with survival (data not shown).

Prognostic Effect of 18q LOH in Strata of Tumor Stage

We examined the prognostic effect of 18q LOH in strata of tumor stage (Table 4). Patient mortality did not significantly differ according to 18q LOH status in any stage strata. The effect of 18q LOH on mortality did not significantly differ according to tumor stage ($P_{\text{interaction}} = .50$ for colorectal cancer-specific mortality and $P_{\text{interaction}} = .76$ for overall mortality).

Prognostic Effect of 18q LOH in Strata of Tumor Location

We examined the prognostic effect of 18q LOH in strata of tumor location (Table 5). The effect of 18q LOH on mortality did not significantly differ according to tumor location ($P_{\text{interaction}} = .17$).

Table 3. 18q LOH Status and Patient Mortality in 532 Stage I to IV Non-MSI-High Colorectal Cancer

18q LOH Status	Colorectal Cancer-Specific Mortality									Overall Mortality								
	Total	Person-			Univariate	Stage-			Multivariate	Person-			Univariate	Stage-			Multivariate	
	No.	Events	Years	HR	95% CI	Matched HR	95% CI	HR	95% CI	Events	Years	HR	95% CI	Matched HR	95% CI	HR	95% CI	
18q LOH negative	184	55	1,404	1	Referent	1	Referent	1	Referent	87	1,404	1	Referent	1	Referent	1	Referent	
18q LOH positive	348	100	2,659	0.96	0.69 to 1.33	1.00	0.71 to 1.41	1.18	0.81 to 1.71	152	2,659	0.92	0.71 to 1.20	0.94	0.72 to 1.24	1.03	0.77 to 1.38	

NOTE. The multivariate, stage-matched (stratified) Cox regression model included age, year of diagnosis, sex, family history of colorectal cancer, tumor location, tumor grade, KRAS, BRAF, PIK3CA, p53, β -catenin, JC virus T antigen, LINE-1 methylation, MSI (low v microsatellite stability), and CpG island methylator phenotype. Abbreviations: LOH, loss of heterozygosity; MSI, microsatellite instability; HR, hazard ratio.

Table 4. 18q LOH and Patient Mortality in Non-MSI-High Colorectal Cancer in Strata of Tumor Stage

AJCC Tumor Stage	Total No.	HR for Colorectal Cancer-Specific Mortality in 18q LOH-Positive Patients*				HR for Overall Mortality in 18q LOH-Positive Patients*			
		Univariate HR	95% CI	Multivariate HR	95% CI	Univariate HR	95% CI	Multivariate HR	95% CI
I to II	262	1.31	0.58 to 2.98	1.24	0.54 to 2.85	0.92	0.58 to 1.47	0.89	0.55 to 1.43
III	160	0.78	0.43 to 1.40	0.76	0.42 to 1.38	0.91	0.56 to 1.47	0.93	0.56 to 1.52
IV	82	0.93	0.58 to 1.48	1.35	0.80 to 2.28	0.89	0.55 to 1.42	1.34	0.80 to 2.25
<i>P</i> for interaction (18q LOH and stage)†		.32		.50		.86		.76	

NOTE. The multivariate Cox regression model included the 18q LOH variable stratified by tumor stage, age, year of diagnosis, sex, family history of colorectal cancer, tumor location, tumor grade, *KRAS*, *BRAF*, *PIK3CA*, p53, β -catenin, JC virus T antigen, LINE-1 methylation, MSI (low v microsatellite stability), and CpG island methylator phenotype.

Abbreviations: LOH, loss of heterozygosity; MSI, microsatellite instability; AJCC, American Joint Committee on Cancer; HR, hazard ratio.

*Versus 18q LOH-negative patients as a referent.

†For assessing a potential interaction, tumor stage was dichotomized as I to II v III to IV.

for colorectal cancer-specific mortality and $P_{\text{interaction}} = .91$ for overall mortality).

Stratified Analysis of 18q LOH and Patient Mortality

We further examined whether the influence of 18q LOH on colorectal cancer-specific and overall mortality was modified by any of the other potential predictors of patient survival (including age, sex, family history of colorectal cancer, BMI, year of diagnosis, tumor grade, and status of CIMP, LINE-1 hypomethylation, *KRAS*, *BRAF*, *PIK3CA*, p53, and β -catenin). None of the strata showed a significant differential effect of 18q LOH on mortality (all $P_{\text{interaction}} > .10$). Notably, the effect of 18q LOH did not significantly differ between the two independent cohort studies ($P_{\text{interaction}} = .89$).

DISCUSSION

We performed this study to examine the prognostic significance of 18q LOH in non-MSI-high colorectal cancer. Because 18q LOH and MSI high are almost mutually exclusive,⁵ and MSI high is associated with better outcome,¹⁷ the association between 18q LOH and poor prognosis might be simply due to the enrichment of MSI-high tumors within 18q LOH-negative cases. Thus it is necessary to examine 18q LOH status in non-MSI-high colorectal cancers to assess the independent prognostic significance of 18q LOH. We found that 18q LOH was

not associated with survival among 532 patients with non-MSI-high colorectal cancer. Moreover, the presence of 18q LOH did not significantly influence patient survival within any individual stratum of pathologic stage or any stratum of clinical and molecular predictors of patient outcome.

Examining prognostic factors is important in colon cancer research.³⁹⁻⁴³ The presence of 18q LOH was associated with recurrence or mortality in colorectal cancer in earlier studies.^{5,8,9,16,44,45} However, many of the previous studies on prognostic significance of 18q LOH^{8,9,12,16} did not consider a confounding effect of MSI. Moreover, a meta-analysis on 18q LOH and outcome in colorectal cancers demonstrated evidence for publication bias.¹⁸ Large studies (total $N > 300$) could be published even with null results,¹³⁻¹⁵ whereas smaller studies were selected for publication based on "significant" results. Smaller studies with nonsignificant results experienced a higher likelihood of being unpublished, leading to publication bias toward a deviation from the null hypothesis.

In the largest study to date, Roth et al¹⁵ failed to show independent prognostic significance of 18q LOH in more than 1,200 patients with stage II and III colon cancer. Our current study ($N = 532$ non-MSI-high tumors), the second largest to date, is in agreement with Roth et al,¹⁵ as well as the two other large studies conducted by Halling et al¹³ ($N = 432$ non-MSI-high tumors) and Barratt et al¹⁴ ($N = 279$ non-MSI-high tumors). Although, in univariate analyses,

Table 5. 18q LOH and Patient Mortality in Non-MSI-High Colorectal Cancer in Strata of Tumor Location

Tumor Location	Total No.	HR for Colorectal Cancer-Specific Mortality in 18q LOH-Positive Patients*				HR for Overall Mortality in 18q LOH-Positive Patients*			
		Stage-Matched HR	95% CI	Multivariate HR	95% CI	Stage-Matched HR	95% CI	Multivariate HR	95% CI
Rectum	138	1.34	0.66 to 2.75	1.46	0.70 to 3.07	1.13	0.64 to 1.98	1.10	0.62 to 1.96
Distal colon, splenic flexure to sigmoid	174	1.30	0.59 to 2.88	1.78	0.78 to 4.05	0.88	0.53 to 1.47	1.01	0.60 to 1.71
Proximal colon, cecum to transverse colon	217	0.87	0.54 to 1.39	0.95	0.57 to 1.58	0.92	0.62 to 1.38	1.01	0.66 to 1.56
<i>P</i> for interaction (18q LOH and location)†		.26		.17		.81		.91	

NOTE. The multivariate, stage-matched (stratified) Cox regression model included the 18q LOH variable stratified by location, age, year of diagnosis, sex, family history of colorectal cancer, tumor grade, *KRAS*, *BRAF*, *PIK3CA*, p53, β -catenin, JC virus T antigen, LINE-1 methylation, MSI (low v microsatellite stability), and CpG island methylator phenotype.

Abbreviations: LOH, loss of heterozygosity; MSI, microsatellite instability; HR, hazard ratio.

*Versus 18q LOH-negative patients as a referent.

†For assessing a potential interaction, tumor location was dichotomized as proximal v distal (including rectum).

Barratt et al¹⁴ found an improved survival with 18q LOH and Roth et al¹⁵ reported an inferior survival in the subset of patients with stage II cancer, both studies found that the prognostic influence of 18q LOH was no longer apparent in multivariate analyses that adjusted for MSI and other relevant parameters. In notable exception, Watanabe et al⁵ obtained informative 18q LOH data in 279 colon cancers and showed that 18q LOH was independently associated with poor outcome. Moreover, Watanabe et al⁵ found a significant relation between 18q LOH and poor survival in stage III non-MSI-high tumors (N = 148), although no such relation was found in stage II tumors. We should be aware that all of these large studies^{5,13-15} (including our study) used different markers and criteria, which could confound the results and cause the somewhat discrepant findings. It would be necessary to develop a consensus panel to assess 18q LOH in colorectal cancer in future trials. Nonetheless, the frequencies of 18q LOH seem relatively comparable between these investigations. Moreover, we demonstrated the significant associations of 18q LOH with JCVT, KRAS, and LINE-1 hypomethylation, supporting the validity of our 18q LOH assessment. LINE-1 hypomethylation and JCVT have previously been related with chromosomal instability.^{29,31,46-49}

We recognize potential limitations in our analysis. Data on cancer treatment in our cohorts were limited. Nonetheless, it is unlikely that use of chemotherapy considerably differed according to 18q LOH status, because such data were not typically available for treatment decision making. In addition, beyond cause of mortality, data on cancer recurrences were not available in these cohorts. Nonetheless, given that the median survival for metastatic colon cancer was approximately 10 to 12 months during much of the time period of this study,³⁷ colorectal cancer-specific survival should be a reasonable surrogate for cancer-specific outcomes.

There are advantages in using the database of the two independent prospective cohort studies, the Nurses' Health Study and Health Professionals Follow-Up Study, to examine prognostic significance of 18q LOH and its relationship with tumoral and host factors. Anthropometric measurements, family history of cancer, other clinical information, pathologic and tumor staging data, and tumoral molecular features were prospectively collected and entered into the database, blinded to patient outcome. Cohort participants who developed colorectal cancer were treated at hospitals throughout the United States and thus were more representative of colorectal cancers in the general population than studies based on a single to few hospitals. Tumor specimen procurement rate has been approximately 60%, and there were no demographic differences between cases with tumor tissue analyzed and those without tumor tissue analyzed.²⁰ In addition, our extensive database of clinical, pathologic, and molecular features enabled us to simultaneously adjust for other predictors of patient outcome.

Beyond the influence of 18q LOH on patient prognosis, there remain limited data on the ability of 18q LOH to predict the benefit from treatment. Among the aforementioned large, published studies that assessed the prognostic significance of 18q LOH,^{5,13-15} only the study by Barratt et al¹⁴ examined whether 18q LOH predicted benefit from fluorouracil-based adjuvant therapy. For patients with LOH at one 18q locus (D18S61), adjuvant fluorouracil did not improve survival, whereas among subjects with no LOH at D18S61, adjuvant fluorouracil did confer a significant survival benefit ($P_{\text{interaction}} = .02$).¹⁴ An ongoing, large clinical trial (Eastern Cooperative Oncology Group Trial 5202) is prospectively examining whether MSI and 18q LOH can define the optimal use of postoperative adjuvant chemotherapy in patients with curatively resected stage II colon cancer. In that study, patients with either MSI-high tumors or non-MSI-high tumors with retention of 18q will not receive postoperative therapy. In contrast, patients with non-MSI-high tumors that demonstrate 18q LOH will receive postoperative chemotherapy (fluorouracil, leucovorin, and oxaliplatin) with or without bevacizumab.

In summary, our large cohort study did not find a significant prognostic impact for 18q LOH in patients with colorectal cancer or any subset of this study population. Future studies are needed to confirm these results.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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