

Chromosomal Instability in Barrett's Esophagus Is Related to Telomere Shortening

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Abstract

Barrett's esophagus is a useful model for the study of carcinogenesis, as the metaplastic columnar epithelium that replaces squamous esophageal epithelium is at elevated risk for development of adenocarcinoma. We examined telomere length and chromosomal instability (CIN) in Barrett's esophagus biopsies using fluorescence *in situ* hybridization. To study CIN, we selected centromere and locus-specific arm probes to chromosomes 17/17p (*p53*), 11/11q (cyclin D1), and 9/9p (*p16* INK4A), loci reported to be involved in early stages of Barrett's esophagus neoplasia. Telomere shortening was observed in Barrett's esophagus epithelium at all histologic grades, whereas CIN was highest in biopsies with dysplastic changes; there was, however, considerable heterogeneity between patients in each variable. Alterations on chromosome 17 were strongly correlated with telomere length ($r = 0.55$;

$P < 0.0001$) and loss of the 17p arm signal was the most common event. CIN on chromosome 11 was also associated with telomere shortening ($r = 0.3$; $P = 0.05$), although 11q arm gains were most common. On chromosome 9p, arm losses were the most common finding, but chromosome 9 CIN was not strongly correlated with telomere length. We conclude that CIN is related to telomere shortening in Barrett's esophagus but varies by chromosome. Whether instability is manifested as loss or gain seems to be influenced by the chromosomal loci involved. Because telomere shortening and CIN are early events in Barrett's esophagus neoplastic progression and are highly variable among patients, it will be important to determine whether they identify a subset of patients that is at risk for more rapid neoplastic evolution. (Cancer Epidemiol Biomarkers Prev 2006;15(8):1451-7)

Introduction

Barrett's esophagus is a hyperproliferative, metaplastic columnar epithelium that replaces the normal squamous esophageal lining as a complication of chronic gastroesophageal reflux disease (GERD; ref. 1). It predisposes to the development of adenocarcinoma of the esophagus; thus, periodic endoscopic biopsy surveillance is recommended for patients with Barrett's esophagus (2), making it an excellent model of human neoplastic progression (3, 4). Progression in Barrett's esophagus has been described as taking place through histologic stages of metaplasia to low-grade dysplasia to high-grade dysplasia to cancer (5). However, interobserver variability in a diagnosis of histology less than high-grade dysplasia complicates its use as a predictor of disease progression (6), and available data suggest that many patients with low-grade and high-grade dysplasia may regress (7-11). The need for reproducible biomarkers to more accurately assess risk of neoplastic progression has spurred investigation of genetic alterations during neoplastic progression in Barrett's esophagus. These have identified, for example, changes in DNA ploidy, *p16* and *p53* in precancerous disease (12-15). It has been suggested that such changes occur during a process of clonal evolution and neoplastic progression (3), which is facilitated by factors that increase chromosomal and genetic instability (16).

Telomere sequences on chromosomal ends afford protection of the chromosome from degradation, recombination, and fusion; loss of this protection by telomere shortening may result in end-to-end chromosomal fusions followed by anaphase bridges, chromosomal breakage, and repetitive bridge-breakage-fusion cycles (17-19). This process can lead to genetic rearrangement, gains and losses, operationally defined as chromosomal instability (CIN; refs. 20, 21). CIN can culminate in DNA aneuploidy, which has been shown by flow cytometry to be a strong predictor of progression from Barrett's esophagus to cancer (14, 15, 22). Flow cytometric aneuploidy must, however, be a late indicator of CIN, as its detection requires a net change in DNA content of at least ~10% as well as clonal expansion of the abnormal cell population. Fluorescence *in situ* hybridization (FISH) allows the identification of earlier stages of CIN that affect single chromosomes or genetic loci in individual interphase cells. CIN has been shown by FISH to be present in esophageal adenocarcinoma and dysplasia (23, 24) and polysomy has recently been reported to be present in metaplastic biopsies (25). In ulcerative colitis, another chronic gastrointestinal disease with increased cancer risk, we have shown that CIN is related to telomere shortening and anaphase bridges (26). In the human breast, telomere lengths have been reported to progressively shorten from atypical ductal hyperplasia to ductal carcinoma *in situ* to invasive cancer, but CIN appeared highest in ductal carcinoma *in situ*, suggesting that telomere-mediated CIN may be most critical in the ductal hyperplasia to ductal carcinoma *in situ* transition in the breast (27). These processes may be relevant to a broad spectrum of precancerous human diseases in which telomere shortening has been observed (28). In this report, we have examined the relationship between CIN and telomere length in Barrett's esophagus.

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Materials and Methods

Biopsies and Histologic Examination. Endoscopic biopsies and surgical specimens for this study are from patients in the Seattle Barrett's Esophagus Study ($n = 66$) and patients with GERD without metaplasia ($n = 15$), sampled as described previously (29, 30). Telomere length was measured in 65 endoscopic and surgical esophageal biopsies from 49 patients with Barrett's esophagus (31 biopsies from 25 patients negative for dysplasia, 10 biopsies from 9 patients indefinite for dysplasia, 8 biopsies from 7 patients with low-grade dysplasia, and 14 biopsies from 13 patients with high-grade dysplasia) as well as from 12 biopsies from 12 patients with adenocarcinoma of the esophagus and, as controls, 23 biopsies from the gastric mucosa of 17 patients with Barrett's esophagus and 15 biopsies from 15 patients with GERD. The average age of patients in the GERD, Barrett's esophagus gastric biopsy, and Barrett's esophagus metaplasia biopsy categories was similar (range, 60-61.1 years), whereas those with higher grades of Barrett's esophagus histology in this study were slightly older (average age range, 65-66.4 years). In a subset of 17 Barrett's esophagus and 2 GERD patients from whom fresh/frozen material was available, both FISH and telomere length measurements were assessed on halves of the same biopsy, including biopsies negative for dysplasia (14 biopsies from 10 patients), indefinite for dysplasia (5 biopsies from 5 patients), low-grade dysplasia (2 biopsies from 2 patients), high-grade dysplasia (4 biopsies from 4 patients), gastric from Barrett's esophagus patients (18 biopsies from 15 patients), and gastric from GERD patients (2 biopsies from 2 patients). The Seattle Barrett's Esophagus Study is approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center with reciprocity from the Human Subjects Division of the University of Washington.

Flow Cytometry and Sorting for FISH. To isolate epithelial cell populations for FISH, fresh/frozen tissue samples were purified by Ki-67/DNA content flow cytometry as described previously (31). Nuclei with a diploid DNA content were sorted into Ki-67-positive and Ki-67-negative populations, which permit separation of epithelial cells from normal stromal cells, as >90% of Ki-67-positive cells in Barrett's mucosa are epithelial (32). For biopsies used for telomere analysis, DNA ploidy was determined from analysis of an immediately adjacent biopsy as described previously (14, 33).

Telomere Length Measurement. Telomere assessment by quantitative FISH was done as described previously (26). Telomere FISH image analysis was done as described previously (34). In brief, the DNA image plane was segmented using a watershed algorithm and the nuclei thus identified were manually indicated by the operator as belonging to either epithelial or stromal categories. Within each nucleus in each category, the green telomere pixel intensity distributions were analyzed using the algorithm shown previously to yield the most reproducible results: the dimmest 20% of green pixels were taken as nonlabeled nuclear background and the mean intensity of this background was subtracted from the mean of the brightest 5% of green pixels.

Fluorescence In situ Hybridization. Approximately 2,000 epithelial cells were sorted by Ki-67/DNA content multivariate flow cytometry as described previously (35) onto plain glass slides in 5 mmol/L CaCl_2 , allowed to dry overnight, and subsequently fixed using 3:1 methanol/acetic acid. FISH was done as described previously (26). An average of 100 nuclei was counted per probe and sample pair. Probes used were *p53* (17p13.1) and CEP17 (centromere), cyclin D1 (11q.13) and CEP11, and *p16* (9p21) and CEP9. Probes were directly conjugated as FITC-centromere/Spectrum Orange-arm locus dual labels (Vysis, Inc., Downers Grove, IL). The number of

FISH spots for each probe was determined by fluorescence microscopy (26); abnormal counts were recorded as loss (less than two spots/probe) or gain (more than two spots/probe). Data are presented as the proportion of cells with abnormal numbers of spots of either color (percentage of cells with CIN) or the proportion of cells in the categories of arm or centromere loss or gain. FISH signals were quantitated only in those biopsies in which signal was clearly above background; 16% of chromosome 11 and 16% of chromosome 9 results were rejected on this basis.

Anaphase Bridges. Anaphase bridge quantification was done as described previously (26) using two primary, mortal epithelial cell cultures from Barrett's esophagus, designated CP-A and CP-C, and the same two cell cultures after transduction with the reverse transcriptase component of human telomerase; these cells and their culture are as described previously (36). The cells were 4',6-diamidino-2-phenylindole stained and cells with $G_2/4N$ DNA content were sorted onto glass slides. For each slide, an average of 1,750 cells was examined by fluorescence microscopy and the frequency of cells with anaphase bridge morphology was enumerated.

Statistical Analyses. Tukey's studentized range test (HSD) was used for comparing the differences of the means of each group in Table 1 and Fig. 2 (the variance ratio test showed that the differences of the variances of each comparison group are not significant). In the data described in Fig. 3, for each of the three types of cells examined (control, Barrett's esophagus negative, and Barrett's esophagus greater than negative histology), five categories of FISH data were observed (arm loss, arm gain, centromere loss, centromere gain, and total instability). These data as percentages were arcsine transformed and Tukey's test was used to compare the difference of the means of each of the FISH categories of the three type cells. In the data of Fig. 4, a single variable linear regression model was used to fit the relationship between relative telomere length and chromosome instability, as nonlinear regression models were found to add no further statistical significance. The slope variable of each linear model was tested to see if it significantly differed from zero. Simple correlation coefficients between relative telomere length and chromosome instability were also calculated for each category of chromosome instability. In assessment of anaphase bridges, the stratified 2×2 table method was used to test the difference of the number of anaphase bridges counted in Barrett's esophagus and human telomerase-transduced Barrett's esophagus cell cultures from two patients. $P = 0.05$ was predetermined to be the statistical significant level. All of the analyses were done with Statistical Analysis System software version 9.0 (SAS Institute, Inc., Cary, NC).

Results

Esophageal biopsies from endoscopy or surgical resection specimens were obtained from the Seattle Barrett's Esophagus Study endoscopic surveillance cohort and analyzed for both CIN using FISH and telomere length using quantitative FISH and confocal microscopy. Only biopsies that were diploid by DNA flow cytometry were used to study earlier stages of CIN.

Table 1. Ratio of telomere to centromere fluorescence (arbitrary units) in Barrett's esophagus and control biopsies

	Epithelium	Stroma
Barrett's esophagus ($n = 11$)	0.4 \pm 0.03	0.81 \pm 0.05
Control ($n = 12$)	0.86 \pm 0.09	1.0 \pm 0.15

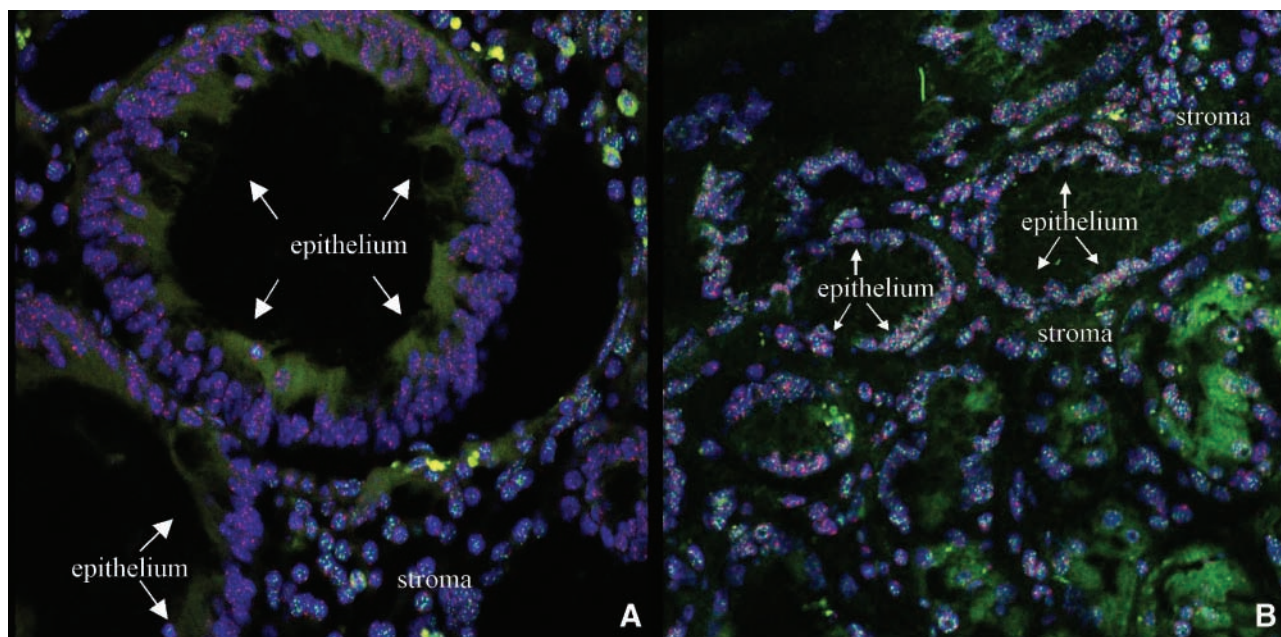


Figure 1. Confocal microscopy of Barrett's esophagus mucosa (A) shows that telomeres are shorter (lower-intensity green fluorescence spots within nuclei) in the epithelial cells compared with the stromal cells that lie between glands. In a gastric biopsy from a non-Barrett's esophagus GERD patient (B), the telomere fluorescence intensity (green nuclear spots) is similar in both cell types. In both panels, centromere fluorescence (red spots) is similar in epithelial and stromal cells. RBC and, to a lesser extent, epithelial cells have green cytoplasmic autofluorescence.

Telomere Shortening in Barrett's Esophagus Epithelium.

Quantitative FISH of biopsies analyzed by confocal microscopy is illustrated in Fig. 1. Green telomere fluorescence was reduced in epithelial cells compared with stromal cells (e.g., endothelial cells, fibroblasts, and leukocytes) in Barrett's esophagus biopsies (Fig. 1A) but not in control gastric biopsies (Fig. 1B). The epithelial/stromal telomere ratio, indicative of relative epithelial telomere length, yielded the most consistent and reproducible measurements, presumably because the stromal cells provide an internal control for the epithelial measurement in each image, making the analysis independent of variations in staining and tissue processing (34).

Figure 2 summarizes the results obtained in each histologic group in the study. Gastric biopsies from GERD patients had an average epithelial/stromal telomere length ratio of 1.10 ± 0.07 (mean \pm SE), whereas the ratio for gastric biopsies from Barrett's esophagus patients was slightly lower (mean \pm SE, 0.86 ± 0.02 ; $P = 0.007$, Tukey's test). The average telomere fluorescence ratio for Barrett's esophagus biopsies negative for dysplasia was substantially lower (0.54 ± 0.04); the difference between Barrett's esophagus biopsies negative for dysplasia and both categories of gastric biopsies is statistically significant (both $P \leq 0.0001$, Tukey's test). Comparison with Barrett's esophagus biopsies of advancing histologic grade (excluding cancer) showed that telomeres were shortest in metaplastic biopsies and progressively increased in length with increasing dysplasia grade ($r = 0.29$; $P = 0.009$). Telomere lengths were yet longer, but highly variable, in adenocarcinoma arising in Barrett's esophagus. Thus, telomere shortening seems to be an early event in Barrett's neoplasia, raising the possibility that it provides an early contribution to CIN and neoplastic progression.

In addition to using stromal cell telomere fluorescence as an internal control, we used a TAMRA-labeled phosphoramidate probe specific for centromere repeats. The centromeres are not vulnerable to the DNA "end replication problem" and should therefore remain relatively constant even if the telomeres are shortened. A confocal image of a Barrett's esophagus biopsy section stained simultaneously with telomere- and centromere-

specific probes (Fig. 1A) illustrates no difference in centromere fluorescence in spite of reduced telomere fluorescence in epithelial (but not stromal) cells. To quantitate this observation, telomere fluorescence intensity was measured relative to centromere fluorescence intensity (telomere/centromere fluorescence ratios) in a randomly selected subset of Barrett's esophagus ($n = 11$) and gastric control ($n = 12$) biopsies. This analysis (Table 1) shows a marked reduction in Barrett's esophagus epithelial cell telomere length in comparison with either Barrett's esophagus stromal, gastric epithelial, or gastric stromal cells (all $P < 0.0003$, Tukey's multiple comparisons test). Telomere lengths in Barrett's esophagus stromal cells and gastric epithelial cells were similar to each other and only slightly (but not significantly; $P > 0.2$) reduced relative to gastric stromal cells (Table 1). Thus, although reduced telomere length in Barrett's esophagus stromal cells cannot be ruled out, if present, it is much smaller in magnitude than that seen in epithelial cells.

CIN in Barrett's Esophagus. To assess CIN in Barrett's esophagus, we used paired dual-colored centromere and locus-specific arm probes to three chromosomes, 17/17p ($p53$; 44 biopsies), 11/11q (cyclin D1; 38 biopsies), and 9/9p ($p16$; 38 biopsies). Using paired arm and centromere probes, two centromere spots and two corresponding arm spots for the same chromosome were considered normal for a diploid nucleus. A count of greater than or less than two spots was considered a gain or loss, respectively, of a centromere or arm.

Abnormal FISH counts (CIN) on chromosome 17 were found to be higher in Barrett's esophagus biopsies with indefinite, low-grade, or high-grade dysplasia (i.e., greater than negative for dysplasia) than in biopsies from gastric controls ($P = 0.00009$) or Barrett's esophagus metaplasia without dysplasia ($P = 0.03$). This was primarily due to 17p arm losses (Fig. 3). Abnormal FISH signals on chromosome 11 were also elevated in indefinite, low-grade, and high-grade dysplastic Barrett's esophagus biopsies compared with gastric controls ($P = 0.006$), with a similar but nonsignificant trend in comparison with Barrett's esophagus metaplasia negative for dysplasia ($P = 0.08$). In contrast to the findings on chromosome 17,

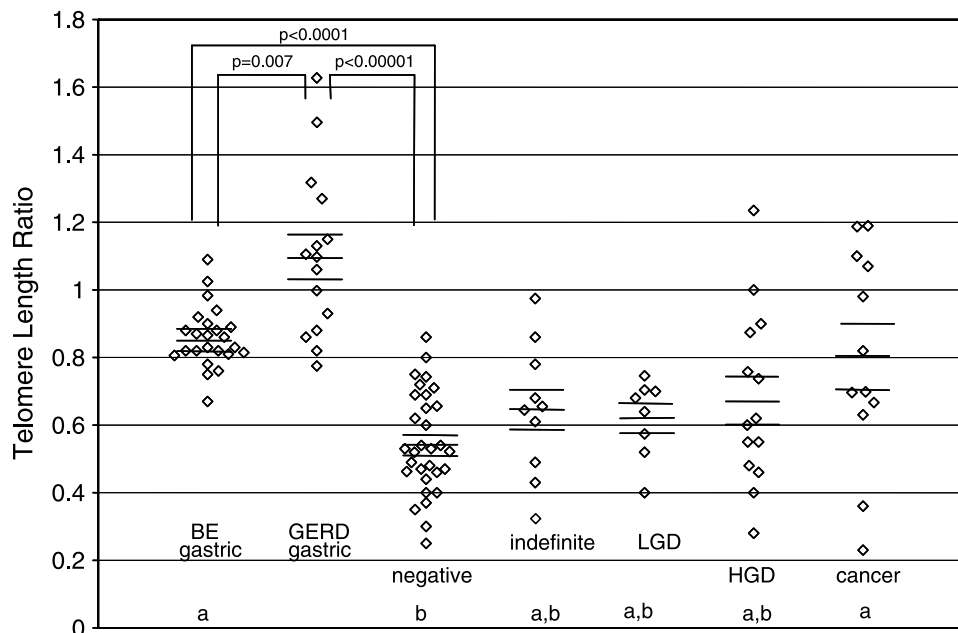


Figure 2. Telomere lengths shorten early in Barrett's esophagus. Telomere lengths were measured as the epithelial/stromal ratio (see text) in gastric epithelium from Barrett's esophagus patients (*BE gastric*) and gastric epithelium from patients with GERD (*GERD gastric*) as well as esophageal biopsies from Barrett's epithelium histologically negative for dysplasia (*negative*) or Barrett's esophagus biopsies that were indefinite for dysplasia (*indefinite*), low-grade dysplasia (*LGD*), high-grade dysplasia (*HGD*), or cancer. Each symbol represents one biopsy with the indicated histology. Bars, mean \pm SE. Bottom, biopsy categories with the same letter (*a* or *b*) are not significantly different from each other (Tukey's test, $P = 0.05$); top, significance of the three most significant pairwise comparisons (Tukey's multiple test).

however, these differences were due primarily to elevated rates of 11q arm gains in Barrett's esophagus biopsies with indefinite, low-grade, or high-grade dysplasia (Fig. 3). Abnormal FISH counts on chromosome 9 were also higher in indefinite, low-grade, and high-grade dysplastic Barrett's esophagus biopsies than gastric control biopsies ($P = 0.006$), and like chromosome 17, this was mainly due to arm losses (Fig. 3).

CIN and Telomere Length in Barrett's Esophagus. We found that decreased telomere length was correlated with increased levels of CIN on chromosomes 17 and 11 but not chromosome 9 (Fig. 4). The most striking correlation was on chromosome 17, which showed a strong correlation between overall CIN and decreasing telomere length ($r = 0.55$; $P < 0.001$). Loss of the arm signal was most common and this was significantly associated with telomere shortening ($r = 0.41$; $P = 0.006$). Centromere loss ($r = 0.54$; $P < 0.001$) and arm gain ($r = 0.43$; $P = 0.006$) on chromosome 17 were less common but were also correlated with telomere length. Chromosome 11 also showed a significant correlation between overall increasing instability and decreasing telomere length ($r = 0.32$; $P = 0.05$); in contrast to observations on chromosome 17, only arm and centromere gains (not loss) showed trends toward associations with telomere length ($r = 0.33$; $P = 0.06$ and $r = 0.33$; $P = 0.08$, respectively). Although instability was present on chromosome 9, only arm loss showed even a trend toward association with telomere length ($r = 0.33$; $P = 0.067$).

Anaphase Bridges. It has been suggested in ulcerative colitis (26), colon cancer (37), and mouse models (38) that telomere dysfunction promotes CIN through cycles of bridge-breakage-fusion. To determine if this mechanism is plausible in Barrett's esophagus, we evaluated the presence of anaphase bridges in two primary cultures of Barrett's esophagus epithelium, because metaphase cells are infrequent in Barrett's biopsies and the size of biopsies is limited. These cell cultures have been shown previously to have shortened telomeres and

high levels of CIN (36). We also examined the same two cell cultures after their transduction with telomerase, which resulted in lengthened telomeres (36). We found that 0.14% to 0.17% of cells from the primary cultures showed visible bridges compared with no apparent bridges in either of the human telomerase-positive Barrett's esophagus cultures ($P = 0.025$ and 0.014 for the two culture pairs, stratified exact test). This result is consistent with previous findings (26, 37, 38) and supports a mechanistic connection between short telomeres and CIN.

Discussion

Analysis of telomere length in Barrett's esophagus by peptide nucleic acid quantitative FISH using confocal microscopy shows that telomeres of epithelial cells are shortened in the earliest stages of Barrett's esophagus. These findings are likely related to the chronic cycles of epithelial regeneration in an environment of chemical damage and inflammation in Barrett's esophagus. These factors promote oxidative damage in Barrett's esophagus (39, 40) and telomere attrition is accelerated by oxidative injury as well as cell proliferation (41, 42). Telomerase activity has been observed to be present at very low levels in the mucosa of Barrett's esophagus, increasing in activity in dysplastic Barrett's esophagus mucosa and cancer (43); it thus seems that low levels of telomerase expression in metaplastic Barrett's esophagus epithelium are unable to counterbalance telomere attrition but that increasing telomerase expression with increasing histologic grade allows the increasing telomere length with grade that we observe.

This study also shows that CIN is related to telomere shortening in Barrett's esophagus. For all three chromosomes examined, CIN was higher in nondysplastic Barrett's esophagus epithelium compared with controls but reached the highest levels in dysplastic biopsies. This result is consistent with the report that CIN is highest in ductal carcinoma *in situ*

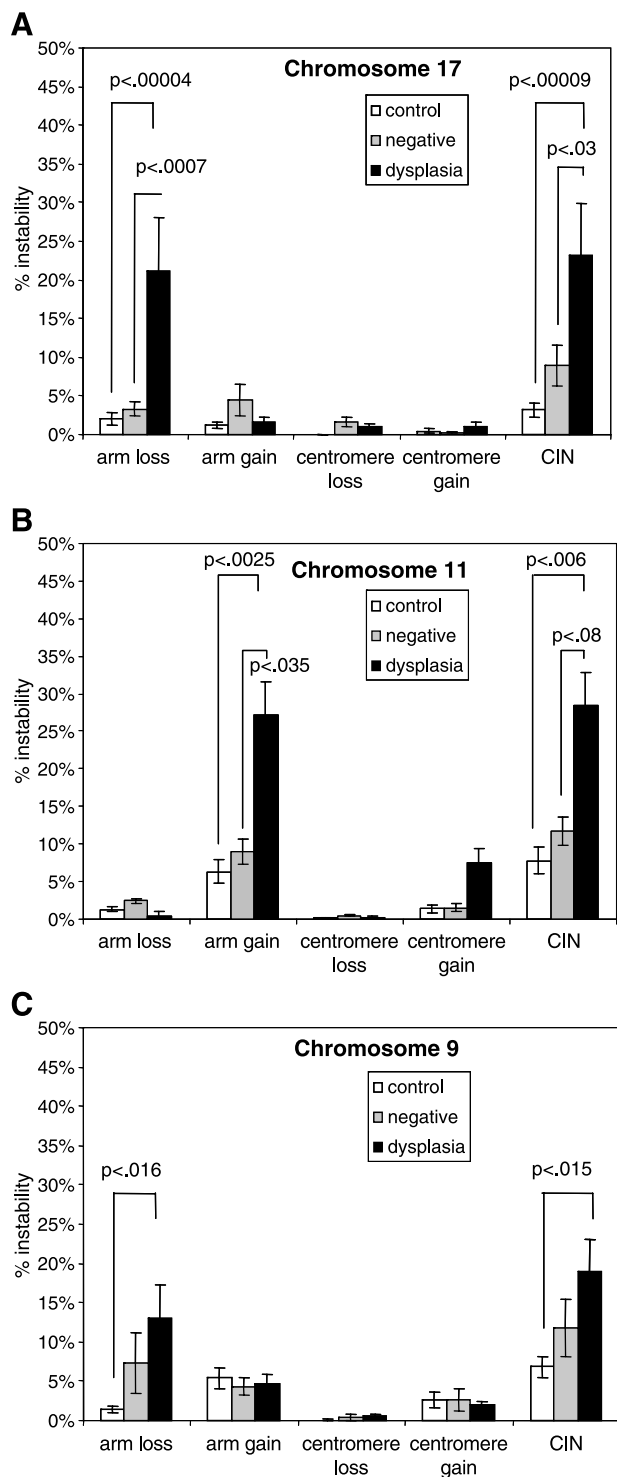


Figure 3. Frequency of cells with CIN (other than two copies each of the chromosomal arm and centromere probe) and subcategories of arm losses and gains, centromere losses and gains, measured by percent of cells showing the respective abnormality. Columns, mean; bars, SE. Compared with gastric controls (white columns), total CIN (any category of FISH abnormality) is increased in metaplastic Barrett's esophagus biopsies negative for dysplasia (shaded columns) but is most elevated in histologically abnormal Barrett's esophagus biopsies (indefinite, low-grade, or high-grade dysplasia; black columns). CIN is manifest predominantly as arm losses on chromosome 17 (A), but it is manifest principally as arm gains on chromosome 11 (B). On chromosome 9 (C), instability that increases with histologic category is primarily evident as arm losses.

of the breast (ref. 27; high-grade dysplasia in Barrett's esophagus being the analogue of ductal carcinoma *in situ*). However, the present study differs from this prior report by clearly indicating that telomere attrition may precede the peak observation of CIN in precancerous human disease; indeed, it seems quite reasonable to hypothesize that bridge-breakage-fusion cycles initiated by telomere shortening early in disease (metaplastic biopsies) can result in the progressive accumulation of chromosomal aberrations that underlie subsequent histologically apparent neoplastic progression.

We found that there is a significant increase in instability in Barrett's esophagus specimens compared with controls for all three chromosomes examined; telomere length was most strongly correlated with instability on chromosome 17 and, to a lesser extent, with instability on chromosome 11 but showed only a nonsignificant correlation on chromosome 9. Furthermore, on chromosome 17, arm loss (17p) is the most common FISH abnormality and is strongly associated with telomere shortening, whereas, on chromosome 11, arm gain (11q) is most common and shows the strongest correlation with telomere shortening.

These loci were chosen because of their known involvement in early stages of Barrett's esophagus neoplasia: inactivation of *p16* (INK4a) by mutation, methylation or loss of heterozygosity (44, 12) loss of *p53* function by mutation and loss of heterozygosity (45, 46), and overexpression of cyclin D1 (24, 47, 48). Alterations at each of these loci are believed to confer a selective advantage to cells during neoplastic evolution and clonal progression. Loss of *p16* occurs in the beginning stages of Barrett's esophagus neoplasia by loss of heterozygosity, mutation, and methylation; losing this CDK4 inhibitor is hypothesized to impart a selective advantage that is permissive for clonal expansion of metaplastic Barrett's epithelial cells (12). Abrogation of *p53* function by loss of one allele and mutation of the other gives cells a survival advantage by removal of cell cycle checkpoints, including cell cycle arrest and apoptosis that can be activated by critically shortened telomeres (49). Overexpression of *cyclin D1* has been observed in premalignant Barrett's esophagus cells (24) and has been reported to be associated with increased risk of progression to cancer (47). Such overexpression is presumed to enhance and/or mimic cellular response to mitogenic growth factors, promoting Rb phosphorylation and cell cycle progression.

Doak et al. have reported results of FISH analysis of cytologic brushings of Barrett's esophagus (25). They also observed increased rates of chromosome 9 arm (*p16* locus) and chromosome 17 arm (*p53* locus) losses in brushings from patients with dysplasia, although the frequency of 17 arm losses that they reported is less than those observed here. This difference may be related to the wide sampling obtained by brushes in comparison with sampling single biopsies, as focal genetic lesions would be diluted in the brushings. Further study is needed to compare the clinical utility of FISH in brushes versus biopsies of Barrett's esophagus.

It is intriguing to speculate as to why different categories of FISH abnormalities are most commonly seen with each chromosome and how this may be related to mechanisms of neoplastic progression in Barrett's esophagus. It is known that CIN is related to the chromosomes with the shortest telomeres (50), and some of the observed FISH differences may be related to variation in telomere length between chromosome arms in Barrett's esophagus; we observed the strongest correlation between telomere shortening and 17p arm loss, and 17p has been shown previously to have the shortest telomeres of all chromosome arms in human peripheral blood lymphocytes (51). However, the striking differences that we observe between telomere length and arm losses on 17p versus arm and centromere gains on chromosome 11 seem more likely to be related to the genetic loci affected. Selective pressure against

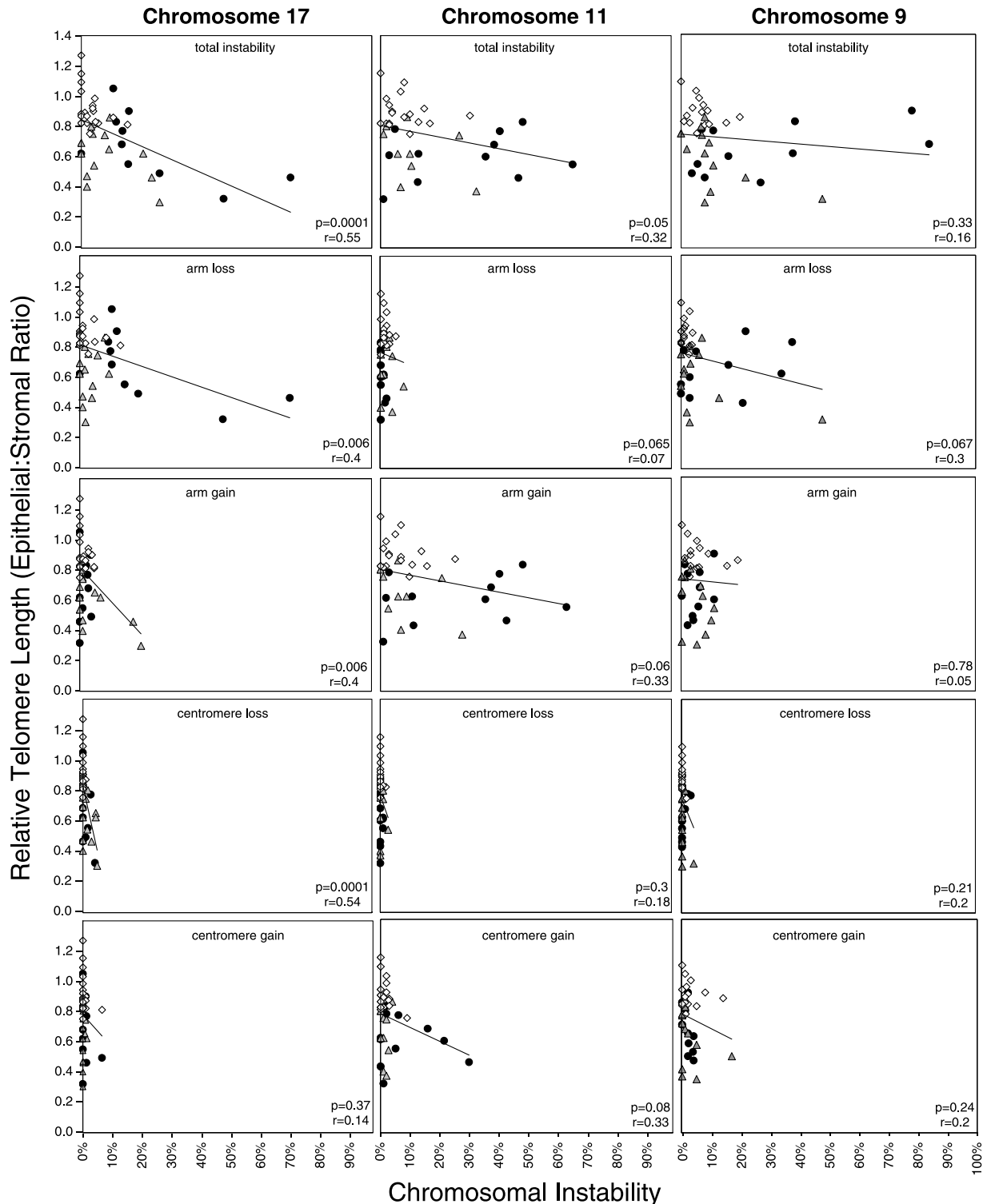


Figure 4. Correlation of CIN (*X* axis; see Fig. 3 legend) versus relative telomere length (*Y* axis) for chromosomes 17 (*left*), 11 (*middle*), and 9 (*right*). CIN was classified as the percentage of cells showing CIN (any category of FISH abnormality; *top row*) and subcategories of arm and centromere losses and gains (*bottom rows*). Individual biopsies were histologically defined as gastric control (*white diamonds*), Barrett's esophagus negative for dysplasia (*gray triangles*), and Barrett's esophagus indefinite, low-grade, and high-grade dysplasia (*black circles*).

p53, located distal on 17p13, would favor arm losses, as telomeres shorten and predispose to chromosome breaks that challenge the p53-mediated checkpoint and apoptosis; this is consistent with the observed losses, greater on the arm than the centromere. Selective pressure mediated by the growth advantage of overexpression of cyclin D1 located near the centromere at 11q13 would favor overexpression; this

would be consistent with our observation of chromosome 11 gains, including a substantial proportion of centromere gains.

Clonal expansions of p16 lesions developing at early stages of neoplastic progression in Barrett's esophagus could subvert cell cycle checkpoint, apoptosis, senescence, and asymmetrical DNA segregation mechanisms that maintain the integrity of intestinal epithelial regeneration (52, 53). This

clonal proliferation could lead to telomere attrition, even in early, nondysplastic stages of Barrett's esophagus, consistent with our observations. Once telomeres shorten to a critical length, CIN would result (50); this seems to be most prevalent once neoplasia has progressed to indefinite, low-grade, or high-grade dysplasia (Fig. 3). The short telomeres on 17p may accelerate CIN and breakage that produces 17p (p53) loss of heterozygosity; this has been associated with progression to increased 4N (G₂/tetraploid) fractions, aneuploidy, and esophageal adenocarcinoma (13, 46). This reasoning suggests that patients with shorter telomeres may be more advanced toward the onset of CIN, and similarly, patients with higher rates of CIN may be more advanced in evolution toward aneuploidy and cancer. As there is substantial variation between patients in both telomere length and CIN at all stages of the disease, further study is needed to determine whether the finding of short telomeres or high levels of CIN identifies a subset of patients that is at risk for more rapid neoplastic evolution. If this is the case, these patients could be targeted for more intensive surveillance or intervention to prevent telomere attrition by decreasing proliferation and oxidative damage.

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