Genomic Alterations in Malignant Transformation of Barrett's Esophagus[1]

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ABSTRACT

The incidence of adenocarcinoma in Barrett's esophagus has been increasing rapidly over the past decades. Neoplastic progression is characterized by three well-defined premalignant stages: metaplasia, low-grade dysplasia, and high-grade dysplasia. A genome-wide overview, based on comparative genomic hybridization, was performed, evaluating 30 Barrett's adenocarcinomas and 25 adjacent precursors, *i.e.*, 6 metaplasias, 9 low-grade dysplasias, and 10 high-grade dysplasias. The frequency of losses and gains significantly increased in the subsequent stages of malignant transformation. Losses of 5q21-q23, 9p21, 17p12–13.1, 18q21, and Y were revealed in low-grade dysplasias. This was followed by loss of 7q33-q35 and gains of 7p12-p15, 7q21-q22, and 17q21 in high-grade dysplasias along with high-level amplification (HLA) of 7q21 and 17q21. In the invasive cancers, additional losses of 3p14-p21, 4p, 4q, 8p21, 13q14-q31, 14q24.3-q31, 16q21-q22, and 22q as well as gains of 3q25-q27, 8q23–24.1, 12p11.2–12, 15q22-q24, and 20q11.2-q13.1 were distinguished along with HLAs of 8p12-p22 and 20q11.2-q13.1. Approximately one-third of the alterations in the dysplasias were also found in the adjacent adenocarcinomas, illustrating that multiple clonal lineages can be present in Barrett's esophagus. Novel findings include loss on 7q, gain on 12p, and the observation of several HLAs in high-grade dysplasias. Furthermore, loss of 7q33-q35 was found to represent a significant distinction between low-grade and high-grade dysplasia ($P = 0.01$), whereas loss of 16q21-q22 and gain of 20q11.2-q13.1 were disclosed to significantly discriminate between high-grade dysplasia and adenocarcinoma ($P = 0.02$ and $P = 0.03$, respectively). This inventory of genetic aberrations increases our understanding of malignant transformation in Barrett's esophagus and might provide useful biomarkers for disease progression.

INTRODUCTION

Barrett's esophagus arises after replacement of squamous epithelium, lining the normal esophagus, with columnar epithelium. This generally occurs in the distal part of the esophagus, probably as a result of gastric refluxes (1–3). It occurs in 11–14% of chronic gastroesophageal reflux disease patients (2). Esophageal adenocarcinoma almost exclusively develops in columnar-lined Barrett's esophagus. Barrett's esophagus is considered a precursor condition for the development of adenocarcinoma, which includes the following successive stages: metaplasia, low-grade dysplasia, and high-grade dysplasia. Over the past decades, the incidence of Barrett-related adenocarcinoma has increased rapidly and is most frequently diagnosed in elderly white males (4). These adenocarcinomas have an extremely poor prognosis, since metastases are frequently present at time of diagnosis (5).

Cytogenetic analyses of Barrett's adenocarcinomas showed frequent losses of chromosomes 4, 18, 21, and Y, and frequent gain of 14 and 20. Frequent rearrangements were seen on 1p, 3q, 11p, and 22q (6). *In situ* hybridization analyses using chromosome-specific centromeric probes demonstrated gains for chromosomes 6, 7, 8, 11, and 12 as well as loss for 17 and Y in these cancers (7–9). Thus far, few comparative genomic hybridization studies have been reported on adenocarcinomas arising at and around the gastroesophageal junction, including Barrett-related adenocarcinoma. Frequent losses were reported on 4pq, 5q, 9p, 14q, 16q, 17p, 18q, 21q, and Y, whereas frequent gain was seen on 1q, 3q, 5p, 6p, 7pq, 8q, 12q, 13q, 15q, 17q, 18p, 20q, and Xpq (10–14). Loss of 14q31-q32.1 was detected in a significantly higher frequency in Barrett-related adenocarcinoma than in gastric cardia cancers (13). Furthermore, we described a subset of shared alterations in a case of multifocal Barrett's adenocarcinoma and adjacent high-grade dysplasia (14). Loss of heterozygosity detected frequent allelic imbalance on 4q, 5q, 9p, 13q, 16q, 17p, and 18q in adenocarcinomas, suggesting involvement of the *APC*[3], *MCC*, *CDKN2A*, *retinoblastoma 1*, *TP53*, and *DCC* genes (15–23). In addition, mutations were revealed in *TP53* and *APC* (18). No mutations were found in *DPC4*, making involvement of *DPC4* unlikely (24). Loss of heterozygosity performed on premalignant lesions showed imbalances at 5q, 17p, and 18q (16, 18, 20, 25). An increasing frequency of the latter imbalances was detected in the successive stages of metaplasia, low-grade dysplasia, high-grade dysplasia, and adenocarcinoma (16). Recently, loss of heterozygosity was used to study evolution of neoplastic cell lineages in Barrett's esophagus, demonstrating that premalignant lesions situated around the tumor consisted of different clonal lineages (26). Protein expression studies have shown that subpopulations of Barrett's adenocarcinoma can have elevated expression of oncogenes *ERBB2* and *EGFR*, whereas only few cases of elevated expression were detected in dysplasia (27, 28). However, activation of the *SRC* oncogene (29) and aberrant expression of *fragile histidine triad* was reported in metaplasias (30). Moreover, polyploidy and aneuploidy have been reported as an early event in Barrett's esophagus (31).

Today, we are not able to predict malignant progression from Barrett's esophagus to cancer. Therefore, it would be useful to gain more insight into the genetics underlying this process by creating an inventory of the alterations in Barrett's esophagus and Barrett's adenocarcinoma by means of a genome-wide overview.

MATERIALS AND METHODS

Patient Material. The study was comprised of 30 Barrett-related adenocarcinomas of the distal esophagus and 25 Barrett's mucosa specimens, adjacent to the adenocarcinomas. The 30 adenocarcinoma samples consisted of 4 stage I, 5 stage IIA, 4 stage IIB, 5 stage III, and 12 stage IV cancers, classified according to the Tumor-Node-Metastasis classification (32). The average age of the group of 30 patients at the time of resection was 65 years, with a male to female ratio of 5:1. The 25 Barrett's esophagus samples consisted of 10 high-grade dysplasias, 9 low-grade dysplasias, and 6 metaplasias. All but two

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[3] The abbreviations used are: APC, adenomatous polyposis coli; MCC, mutated in colorectal cancer; TP53, tumor protein p53; DCC, deleted in colorectal carcinoma; EGFR, epidermal growth factor receptor; ERBB2, v-*erb*-b2 oncogene 2; SRC, v-src avian sarcoma oncogene.

samples were collected by manual microdissection of Formalin-fixed, paraffin-embedded tissue sections. The use of archival, Formalin-fixed tissue sections allowed for a precise selection of representative tumor and adjacent preneoplastic areas. Microdissection of the selected areas was performed by scraping successive 10-μm tissue sections. Before microdissecting, the slides were deparaffinated using standard methods and stained for 30 s with hematoxylin. After dehydration by an ethanol series, the slides were treated with 2.5% glycerol for 30 s to facilitate dissection. Scraping was performed using a stereo microscope and a 0.4- $\times$ 12-mm hollow needle. The presence of the lesion was confirmed on H&E-stained tissue sections of the upper and lower boundaries. Two fresh-frozen samples (two metaplasias) were laser microdissected and the DNA was randomly amplified by degenerate oliogonucleotide-primed PCR (33).

DNA Isolation and Labeling. Isolation of DNA from the Formalin-fixed, paraffin-embedded material was performed using standard procedures. Test DNA with fragment size $<$1 kb was labeled using the Universal Linkage System biotin labeling kit (Kreatech Diagnostics, Amsterdam, The Netherlands) according to the manufacturer's directions (34). Test DNA with larger DNA fragment sizes was labeled with biotin by nick translation (Nick Translation System; Life Technologies, Inc., Gaithersburg, MD). Likewise, male reference DNA (Promega Corporation, Madison, WI) was labeled by nick translation with digoxigenin (Boehringer Mannheim, Indianapolis, IN).

Comparative Genomic Hybridization. Comparative genomic hybridization was essentially performed according to the procedure described by Kallioniemi *et al.* (35) with some modifications (34). Images were acquired with an epifluorescent microscope (Leica DM; Leica, Rijswijk, The Netherlands) equipped with a charge-coupled device camera (Photometrics Inc., Tucson, AZ), three single excitation filters, a multiband pass dichroic mirror, and emission filters. For comparative genomic hybridization analysis, Quips XL software from Vysis (version 3.1.1; Vysis Inc., Downers Grove, IL) was used. Loss of DNA sequences was defined as chromosomal regions where the mean green to red ratio was below 0.85, while gain was defined as chromosomal regions where the ratio was above 1.15. These values were based on series of normal controls (34). Furthermore, each hybridization included a separate normal male *versus* female control. Approximately 90% of all paraffin samples were analyzable; five samples were repeated to confirm results. A high-level amplification probably representing an amplicon was seen as a distinct peak (ratio above 1.5). At least 8–10 metaphases were used for the analysis of gains and losses per sample. The data were collected in chromosomal ideograms. Assignment of candidate genes to smallest regions of overlap was done by screening the genecards database of the Weizmann Institute[4] (36).

Statistical Analysis. Chromosomal regions were subjected to statistical analysis if their frequency of imbalance for loss or gain was $\geq$25% in the adenocarcinoma group. This threshold was empirically chosen at a level where "background" is avoided. Percentages of aberrations between low-grade dysplasia and high-grade dysplasia, as well as high-grade dysplasia and adenocarcinoma, were evaluated using Fisher's exact test, after it was verified that there were no statistical associations within patients between the results found in adenocarcinoma and in premalignant lesion. Comparison of the subsequent stages of tumorigenesis without (metaplasia, low-grade dysplasia, and high-grade dysplasia) and with adenocarcinoma (metaplasia, low-grade dysplasia, high-grade dysplasia, and adenocarcinoma) regarding prevalences of various characteristics was done using the χ^2 test for trend of percentages. Correlation coefficients (r_s) given are Spearman's rank tests. $P = 0.05$ (two sided) was considered to be the limit of significance.

RESULTS

Comparative genomic hybridization was performed on 30 Barrett-related adenocarcinomas and 25 adjacent Barrett epithelia, *i.e.*, 6 metaplasias, 9 low-grade dysplasias, and 10 high-grade dysplasias (Table 1). The observed aberrations were collected in separate ideograms, which are shown in Fig. 1. In all 30 adenocarcinomas, multiple alterations were observed (average of 11.0 altered chromosomes per tumor). Frequent loss ($\geq$25% of the tumors) was detected, in decreasing order of frequency, on 18q (63%), Y (60%), 9p (53%), 4p (47%), 4q (40%), 16q (40%), 7q (37%), 14q (37%), 13q (33%), 17p (30%), 3p (30%), 22q (30%), 5q (27%), and 8p (27%). Frequent gain ($\geq$25% of the tumors) was seen, in decreasing order of frequency, on 20q (53%), 8q (47%), 7p (37%), 7q (37%), 15q (30%), 3q (27%), 12p (27%), and 17q (27%). Many high-level amplifications were disclosed: five at 7q21 and 8p12-p22, three at 10q21.3-q25 and 20q11.2-q13.1, two at 17q21, and one at 1q21, 3q26.3, 8q24.1, 12p11.2, and 15q22-q24. One patient displayed three different high-level amplifications within one adenocarcinoma, whereas two other patients showed two within the same cancer. Minimal overlapping regions were assigned to pinpoint areas where putative oncogenes or tumor suppressor genes reside. Minimal overlapping regions for loss in the adenocarcinomas were 3p14-p21, 5q21-q23, 7q33-q35, 8p21, 9p21, 13q22–32, 14q24.3-q31, 16q21-q22, 17p12-p13.1, and 18q21. Minimal overlapping regions for gain were 3q25-q27, 7p12-p15, 7q21-q22, 8q23-q24.1, 12p11.2-p12, 17q21, and 20q11.2-q13.1.

No alterations were detected in metaplasia by comparative genomic hybridization. The dysplasias were then screened with emphasis on aberrations seen frequently in the adenocarcinomas (see above). An average of 3.2 altered chromosomes per sample was observed in low-grade dysplasia, whereas in 2 of the 9 low-grade dysplasias no alterations were found. Frequent loss on relevant chromosome arms was detected on 5q (22%), 9p (33%), 17p (22%), and Y (22%). No recurrent gains were seen in low-grade dysplasias. Multiple alterations, with an average of 7.6 altered chromosomes per sample, were found in all 10 high-grade dysplasias. Frequent loss was observed, in decreasing order of frequency, on 18q (70%), 7q (60%), 5q (60%), Y (40%), 9p (40%), 17p (40%), and 12q (30%). Frequent gain was seen on 13q (40%), 7p (30%), 7q (30%), and 17q (30%). In addition, three high-level amplifications were detected: two on 7q21.3 and one on 17q21. The following minimal overlapping regions were found in dysplasia: 5q21-q31, 7q33-q35, 9p21, 17p12-p13.1, and 18q21 could be assigned for losses, whereas for gains 7q21-q22 and 17q21 were revealed.

A gradual increase was found in the mean number of aberrant chromosomes, chromosomes showing loss and chromosomes showing gain in the successive stages of metaplasia, low-grade dysplasia, high-grade dysplasia, and adenocarcinoma (Spearman's rank test, all $P < 0.001$; see Fig. 2). Significant trends regarding increased prevalences in Barrett's esophagus (metaplasia, low-grade dysplasia, and high-grade dysplasia sequence) were loss of 5q21-q31 ($P = 0.01$), 7q33-q35 ($P < 0.01$), and loss of 18q21 ($P < 0.01$). Extension of the sequence with the adenocarcinomas revealed significant trends for increased prevalence of loss of 4p ($P < 0.01$), 4q ($P = 0.02$), 7q33-q35 ($P = 0.03$), 9p21 ($P = 0.02$), 13q14-q31 ($P = 0.01$), 14q24.3-q31 ($P < 0.01$), 16q21-q22 ($P < 0.01$), 18q21 ($P < 0.01$), 22q ($P = 0.01$), and Y ($P < 0.01$). Likewise, significant trends for gain were seen on 7p12-p15 ($P = 0.03$), 7q21-q22 ($P = 0.03$), 8q23-q24 ($P < 0.01$), 12p11.2-p12 ($P = 0.03$), and 20q11.2-q13.1 ($P < 0.01$) and for high-level amplification ($P < 0.01$). About one-third of the aberrations present in the dysplasia were also found in the adjacent carcinoma (see Table 1). Only patients 22 and 24 had conserved all aberrations of the premalignant lesions in the adjacent adenocarcinomas. These findings illustrate that premalignant lesions surrounding the adenocarcinoma consist of several different clonal lineages (26). Furthermore, all three high-level amplifications detected in high-grade dysplasia could also be distinguished in the adjacent adenocarcinoma. Comparison of increasing frequencies of high-grade dysplasia and adenocarcinoma revealed two significant differences, *i.e.*, loss of 16q21-q22 ($P = 0.02$) and gain of 20q11.2-q13.1 ($P = 0.03$). In addition, a significant increase was observed between low-grade dysplasia and high-grade dysplasia for loss of 7q33-q35 ($P = 0.01$).

[4] World Wide Web URL: http://bioinfo.weizmann.ac.il/cards.

Table 1 *Chromosomal aberrations identified by comparative genomic hybridization*

Patient	Sex	Age	Pathology[a]	LOSS[b]	GAIN[b]
1	F	73	Adenocarcinoma T3N0M0G2R1	-1p13-p31;-3p12-pter; -8p21-pter; -9; -10q21; -11p13-pter; -17p11.2-p12; -18q11.2-qter; -22q11.2-qter	+2p14-p16; +20q11.2'-13.2; HLA20q12
2	M	64	Adenocarcinoma T3N1M1G3R1	-3p12-pter; -6p22-pter; -7q33-q34; -14q24-q31; -15q22-q24; -16q12.1-q22; -17p11.2-p12; -18q12-q21	+3q25-qter; +6q12-q23
3	M	50	Adenocarcinoma T3N1M1G3R1	-4p15.1-pter; -9p22-pter; -18q12-qter; -22q11.2-qter; -Y	+7p11.2-p21.2; +7q11.2-q22; HLA7q21.3; +13q31.1; +20q11.2-q13.2
			M	NA	NA
4	M	85	Adenocarcinoma T3N1M1GxRx	-1q32-qter; -4; -10; -14q31-qter; -16q12.1-qter; -Y	+7q21-q35; +8p21-p22; +8q22-qter; HLA8q24.1; +9p12-p23; +12p11.2-p12; +12q12-q24.2; +15q11.2-qter; HLA15q22-q24
5	M	70	Adenocarcinoma T4N1M1G2R1	-3p14-p24; -5p15.1-p15.2; -7q33-q35; -8p12-p21; -9p12-p23; -9q13-q22; -11p15.2-p15.3; -14q23-qter; -15q11.2-q25; -22q11.2-qter	+2q24-q31; +7q11.2-q22; +8q11.2-qter; +13q21-q22; +17q11.2-q22; HLA 17q21; +18p11.2-pter; +18q11.2; +20q11.2; +Xq25-qter
6	M	74	Adenocarcinoma T3N1M1G3R0	-9p12-p23; -9q13-q21; -Y	+1q21-q22; +8q22-qter; +10q23-q24; +12q15-qter; +15q21-qter; +20
7	M	73	Adenocarcinoma T1N0M0GxR0	-4; -17p12.1-pter; -18q12-qter; -Y	+3q25-q27; HLA3q26.3; +6p12-p21.2; +17q21
8	M	68	Adenocarcinoma T3N1M1GxR0	-5p15-; 8p21-pter; -10p12-pter; -Y	+1q21-q22; HLA1q21; +5q33-q34; +8q23; +9q31-q32; +11q12-q22
9	M	44	Adenocarcinoma T1N1M0G2R0	-10p13; **-13q31-q32**; **-17p12**; **-18q21-q22**; **-Y**	+3q12-q13.2; +3q24; +4q22-qter; **+7p14-p15**; **+7q11.2-q31.1**; **HLA 7q21**; +18p11.2-pter
			HGD	-5q21-qter; -7q33-34; -9p13-pter; -12q15-qter; **-13q21.3-qter**; **-17p11.2-pter**; **-18q12.3-qter**; **-Y**	+1p12-p31; +1q21-q25; +2p13; **+7p13-p21.1**; **+7q11.2-q22**; **HLA7q21.3**; +13q13-q14.1
10	F	52	Adenocarcinoma T1N0M0G3R1	-3p21-p25; -4p14-pter; **-7q32-qter**; -8p11.2-pter; -13q21-qter; -18q21-q22; -22q11.2	+7p11.2-p14; +8q21.3-q23; **+10q23-q24**; +20p11.2-p12; +20q11.2-qter
			HGD	-1p36.1-pter; **-7q33-qter**; -14q24-qter	+3p12; +6q21; **+10q21-q25**; +18p11.2-pter
11	M	64	Adenocarcinoma T2N1M0G3R0	-3p13-p24; -9p13-p23; -13q31-q32; -Y	**+7q11.2-q22**; **HLA 7q21.2**
			HGD	-2q36; -5q31-q34; -9q34.1; -12q24.1; -17p12	+6q12-q22; +7p15-7p21; **+7q11.2-q22**; **HLA7q21.3**; +13q21
12	F	67	Adenocarcinoma T3N1M1G2R0	-4p14-p15.1; **-9p12-pter**; -13q14-q21; **-18q12-qter**	+3q24-qter; +8q11.2-qter; +10q24-qter; +12p11.2-pter
			HGD	**-9p13-p22**; -12q14-qter; **-18q12-qter**; -20p11.23-pter	+17q21; +17q24
			M	NA	NA
13	M	53	Adenocarcinoma T3N0M0G3R0	**-4**; **-5q13-qter**; -13q12-qter; -14q11.2-qter; -16q12.1-qter; -22; **-Y**	+6p12-p22; +9p12-p23; **+9q22-qter**; +12q21-q23; **+15q13-q21**; +19p13.1; +19q13.1-qter; +20p11.2-p12; **+20q11.2-qter**
			HGD	**-4q31-qter**; **-5**; -7p14-p15; -7q22-qter; -10p14-pter; -11p14-pter; -12q21.1; -15q11.2-q14; -17p11.2-p12; -18q12-qter; **-Y**	+4q28; +8p12; **+9q21-qter**; +10q21.3; +10q24-qter; +11p11.2; +11q12; +13q22-qter; **+15q21.2-q25**; +16p11.2-p13.2; +16q23-qter; +17q11.2; +17q21-q24; **+20q12-q13.1**
14	M	68	Adenocarcinoma T2N1M0G3R0	-18q11.2-qter; -Y	+15q24-qter
			HGD	NA	+16p11.2-pter
15	M	76	Adenocarcinoma T2N0M0G3R0	-4; **-5q21-q32**; -6p21.3-p23; -7q32-q34; -14q22-q31; -16p11.2-p13.1; -16q12.1-q23; **-17p11.2-p12**; -21q21	+13q14-q21; +15q22-qter; +X
			HGD	-2p22-p23.2; -2q33-q35; **-5q31-q34**; -11p14-p15.1; **-17p11.2-p12**; -18q12.3-q21	NA
16	M	49	Adenocarcinoma T3N1M0G2R1	-2q21-q31; **-3p12-pter**; -4; **-5q11.2-q23**; -6q12-q25; **-7q32-qter**; **-9p21-pter**; -14q11.2-q31; -15q11.2-q24; -16q12.1-qter; **-18**; -21q11.2-q21; **-Y**	+5p15.2-pter; **+7p11.2-pter**; +7q11.2-q21; +8q12-qter; +9q13-qter; +16p12-pter; +17; HLA17q21.2; +20q11.2-q13.1
			HGD	**-3p22-p25**; -4; **-5q23-q34**; **-7q33-7q35**; -8p21-8p22; **-9p21-9p22**; **-18p11.2-18pter**; **-18q11.2-18qter**; **-Y**	+5p12-pter; **+7p21**; +13q12-qter; +17q12-q21; HLA17q21; +Xp11.2-p21; +Xq12-qter
17	M	53	Adenocarcinoma T3N1M1G2R1	-4q32-qter; -14q24.3-qter; **-18q21**	+1q21-q31; +5p12-pter; +7p11.2-p14; +12q21;+13q22-qter; +18p11.2-pter
			HGD	-7q32-q35; -15q12-q14; **-18q12.3-q21.1**	+X

Table 1 *Continued*

18	M	65	Adenocarcinoma T3N1M0G3R0	-4p12-p14; -4q12-qter; -5p12-p13; **-5q11.2-qter**; **-9p12-p23**; **-9q13-q33**; **-18q12-qter**; **-Y**	+1q21-q25; +1q32.2-q42; +3q21-qter; +6p21.1-p22; +7p11.2-pter; **+8p21pter**; HLA8p22; +8q23-qter; +10q21-q22; **+12p11.2-pter**; +15q22-q25; +16q21-q23; +17q21-q23; +20q12-qter
			HGD	**-5q13-q22**; -7q33-q35; **-9p12-p23**; **-9q13-q22**; **-18q12-qter**; -21; **-Y**	+7q11.23; **+8p21-pter**; +8q11.2-q21.1; **+12p11.2-pter**; +Xp11.2-p21; +Xq12-qter
19	F	78	Adenocarcinoma T3N1M1G2R1	-4p12-p15.1; -5q12-qter; -6p23-pter; -6q14-q22; -7q31.3-qter; -8q13; **-9p21-p23**; -10p11.2-pter; -10q11.2; -10q23.2-qter; -13q22-qter; -14q21.3-qter; -15q13-q24; -16q13-qter; -18q12-qter	+2p13; +3q21; **+7p12-p21.1**; +8p12-pter; HLA8p21.3; 11q23-qter; +20q11.2
			LGD	-2p22-pter; -5q13-qter; **-9p13-p21**; -17p11.2-pter	+1p21-p22; +3q23-q26.2; **+7p11.2-p15**; +7q11.2-qter; +15q21-qter; +16q23-qter
20	M	73	Adenocarcinoma T2N1M1G3R0	-1p13-p31; -1q24-q32.1; -2p21-p23; -4p12-p15.3; -4q12-qter; -6q12-q22; -7q21-q31; -8p11.2-pter; -8q11.2-q13; -16q12.1-qter; -18; -Y	+2q11.2-q13; +8q21.2-qter; +9q22-qter; +12pter; +12q22-q24.2; +12q13; +15q11.2-q24; +16p11.2-p13.2; +17q11.2-qter; +19; **+20q11.2-qter**HLA
			LGD	NA	+8q13; **+20q11.2-q13.2**
21	M	74	Adenocarcinoma T3N0M0G2R0	-1p32-pter; -6q25-qter; -7q32-qter; -8p12-pter; -9p12-pter; -9q13-q21; -10p11.2-pter; -12q22-qter; -14q21-qter; -15q14-qter; -17p11.2-pter; -21q11.2-qter; -22q11.2-qter	+1q32.2-qter; +4p12-pter; +4q12-q13; +5p12-p15.2; +6p11.2-p21.1; +6q16-q21; +7p15; +7q21-q31; HLA7q22.2; +8q21.1-q22; +10q21-q22; HLA10q21.3; +11p11.2-p14; +12p11.2-p12; +12q14-q15; HLA12p11.2
			LGD	NA	NA
22	M	63	Adenocarcinoma T3N1M0G3R1	-5q23-q34; -6q24-qter; -9; -10q21-q23; -11p14-pter; -11q14-qter; -13q12-qter; **-16q21-q23**; -17p11.2-p12; -18q11.2-qter; **-21q11.2-qter**	+2p23-p24; +2p14-p16; +2q31; +7p13-pter; +8p21-p22; HLAp21.3; +10q24-q25; HLA10q25.1; +12p11.2-pter; +14q22-qter
			LGD	**-16q13-q21**; **-21q21**	NA
23	M	51	Adenocarcinoma T1N0M0G2R0	-2p21-p22; -7q32-q35; -9p21; -10q23-q25; -11p15.1-pter; -13q22-qter; -14q24.3-qter; -18q21-q22; -22q11.2-qter; -Y	+3q25-qter; +7q11.2-q22; +8p11.2-pter; HLA8p21.3
			LGD	NA	NA
24	F	48	Adenocarcinoma T3N1M0G3R1	-2p22-p23; -2q33-q34; -4p13-p15.2; -4q31.3-q32; -5p14; -5q21; -7p11.2-p21.2; -7q31-q35; -9p12-p23; -11q14; **-12q15-q23**; -13q14-q31; **-18q12.3-q21**; -21q11.2-q21	+7q11.2; +7q22; +8q11.2-q22; +17q11.2-q21
			LGD	**-12q23**; **-18q21**	NA
25	M	70	Adenocarcinoma T3N0M0G2R1	-16; **-17**	+5p12-pter; +8p11.2-p12; +8q11.2-q23.3; HLA8p12; +20p11.2; +20q11.2-qter; **+X**
			LGD	-3p24-3p25; **-17p11.2-17p12**; -Y	+11q23; **+X**
26	M	61	Adenocarcinoma T3N1M0G3R0	-16; -19q13.1-q13.2; -22q11.2-qter; **-Y**	+6q16-q22; +8q21.2-q23; +10p11.2-pter; +10q11.2-q22; +10q25-qter; HLA10q25; +13q14-q32; +20
			LGD	-4; -5; -9p12-pter; -11q22-q23.2; -18q11.2-q21; -20q11.2-q13.1; **-Y**	NA
27	M	63	Adenocarcinoma T1N0M0G2R0	-3p13-p21; **-8p12-pter**; -14q22-qter; -17p12-pter; -18q12-q21; -Y	+5p15.1-p15.2; +7p11.2-p21.2; +7q11.21-qter; +13q21-qter; +20q11.2-q13.1; +Xp11.2-p21; +Xq12-qter
			LGD	**-8p22**; -9p12-p23; -16p11.2-p12	NA
28	M	70	Adenocarcinoma T1N1M0G3R0	-1p31.2-p34.3; -3p14-pter; -4p12-pter; -4q31.3-qter; -5q13-q21; -6q24-qter; -7q22-qter; -8p11.2-pter; -8q11.2-q13; -9p12-pter; -10q11.2-q23; -11p14-pter; -16q12.1-qter; -18; -19; -21; -22; -Y	+1p12-p22; +1q21-q23.1; +2p13.2-p21; +5p12-p15.2; +5q11.2; +7p12-pter; +8q21.3-qter; +12p11.2-pter; +12q12-q15; +12q23-qter; +14q12-q21; +15q21-qter; +16p13.1; +20q11.2-qter; HLA20q13.1
			M	NA	NA
29	M	67	Adenocarcinoma T3N1M1G3R1	-1p22-p31.1; -3p12-pter;-4p12-pter; -4q13-q27; -4q32-qter; -6q16-qter; -9p12-pter; -11q23-qter; -13q21-qter; -16q12.1-qter; -18q11.2-qter; -21q11.2-qter; -Y	+3q12-q27; +6p21.1-p22; +7q11.2-q22; HLA7q21.3; +12p11.2-pter; +15q15-q21; +16p11.2-p13.2; +17q11.2-q24; +20q11.2
			M	NA	NA
30	M	69	Adenocarcinoma T3N1M1G3R0	-4q12-qter; -9p13-p23; -Y	+16p11.2-pter; +17; +20
			M	NA	NA

[a] Adenocarcinomas were classified according to the Tumor-Node-Metastasis classification (32); HGD, high-grade dysplasia; LGD, low-grade dysplasia; M, metaplasia.
[b] Bold print, aberration identified in both adenocarcinoma and dysplasia of the same patient; NA, no aberrations detected.

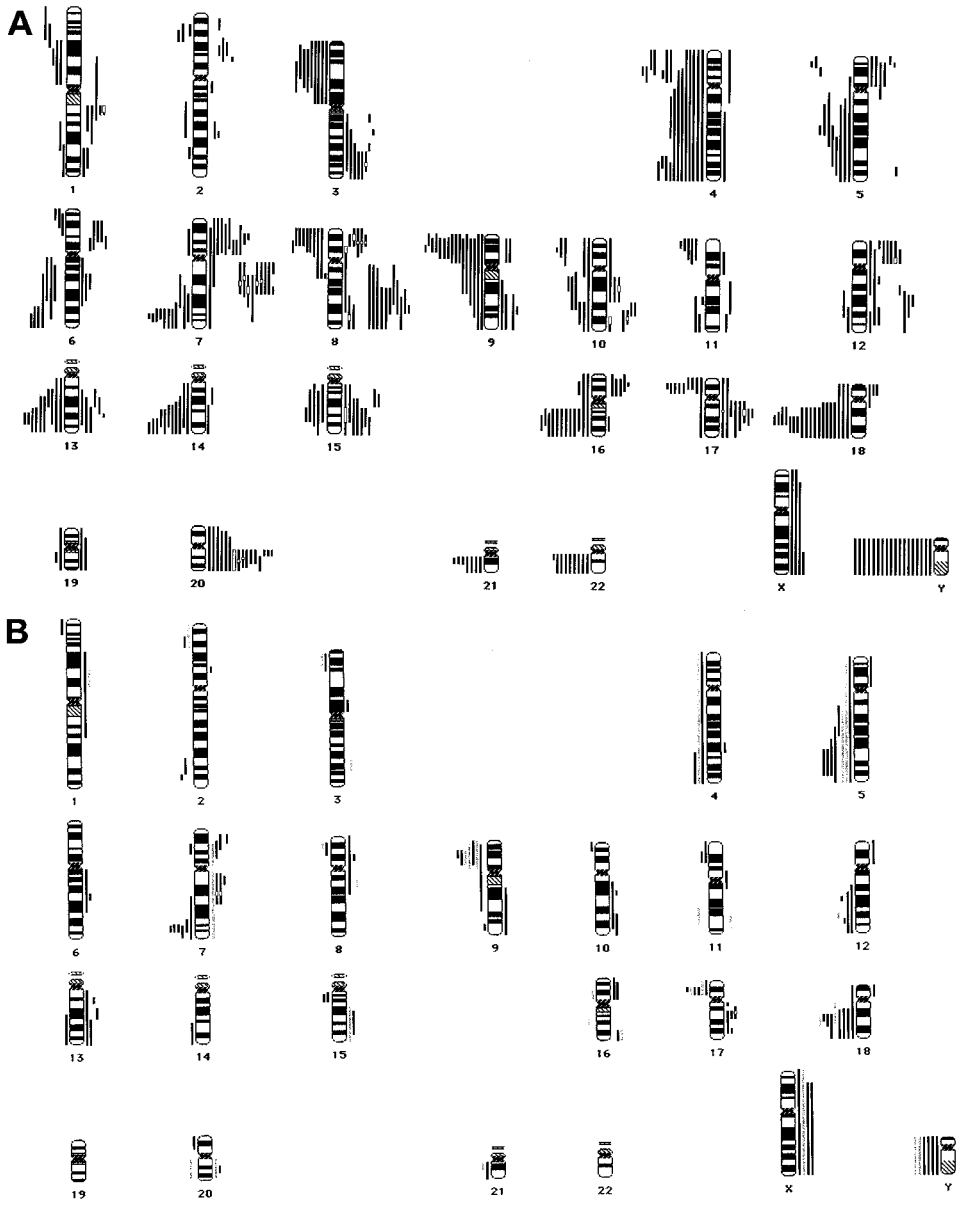

Fig. 1. Losses and gains were collected in chromosomal ideograms to summarize DNA copy number changes detected by comparative genomic hybridization. A *bar* on the *left side* of the ideogram represents loss; a *bar* on the *right side* represents gain of a chromosomal region. *Open boxes* within *bars* represent high-level amplifications. Chromosomal ideograms were constructed for adenocarcinomas ($n = 30$; *A*) and dysplasias ($n = 19$; *B*), the latter consisting of 10 high-grade dysplasias and nine low-grade dysplasias. No alterations were seen in metaplasia. *Black bars* in *B*, aberrations of the high-grade dysplasias; *gray bars*, aberrations of the low-grade dysplasias.

DISCUSSION

The cancer-related genes *fragile histidine triad*, *CTNNB1* (β-catenin), *APC*, *MCC*, *CDKN2A*, *CTSB* (cathepsin B), F37/esophageal cancer-related leucine-zipper motif (*FEZ1*), *retinoblastoma 1*, *CDH1* (E-cadherin), *TP53*, *DCC*, *EGFR*, *SRC*, and *ERBB2* are located within the smallest regions of overlap on frequently altered chromosome arms discriminated in this study. Moreover, these genes showed allelic imbalances, mutations, or altered expression in Barrett-related adenocarcinoma (15, 18, 22, 24, 25, 27, 29, 30, 37–41). Other candidate genes, such as caspase 2 (*CASP2*), tumor necrosis factor-related apoptosis-induced ligand receptor B (*TNFRS10B*), *TSHR*, v-Ki-*ras2* Kirsten rat sarcoma 2 viral oncogene (*KRAS2*), *hepatocyte growth factor*, v-*myc* myelocytomatosis (*MYC*), and insulin-like growth factor 1 receptor (*IGF1R*), can be selected based on location and oncogenic or tumor-suppressive potential in general, *e.g.*, *TNFSR10B* is of interest, since it is expressed in the proliferating compartment of colonic crypt mucosa and loss of this locus has been reported for colon cancer (42). Several members of the cadherin gene family, *e.g.*, *CDH1* (E-cadherin) are located on 16q21-q22 (43). Reduced expression levels of proteins involved in the E-cadherin-catenin complex have been described in Barrett's esophagus and adenocarcinoma (37, 41). Reduced levels of E-cadherin, α-catenin, and β-catenin in Barrett-related adenocarcinoma correlated with a poor prognosis (37, 41). Our comparative genomic hybridization showed frequent losses in the vicinity of *CDH1* (16q22) and *CTNNB1* (3p21.3-p22), the latter coding for β-catenin. In addition, aberrant copy numbers were detected of gene loci harboring genes like *APC*, *EGFR*, *IGF1R*, and *SRC*, which can influence maintenance and expression levels of proteins involved in the cadherin-catenin complex (43). It has been shown that subpopulations of Barrett's adenocarcinoma have elevated or overexpression of ERBB2 (27, 28), possibly a gene dosage effect. Although a high frequency of loss is observed on chromosome 4, it was not possible to assign a smallest region of overlap. This could be due to a high density of involved tumor suppressor gene loci on chromosome 4, which is supported by a recent loss of heterozygosity study. The authors described that at least three loci on the long arm of chromosome 4 were frequently altered in Barrett's adenocarcinoma (17).

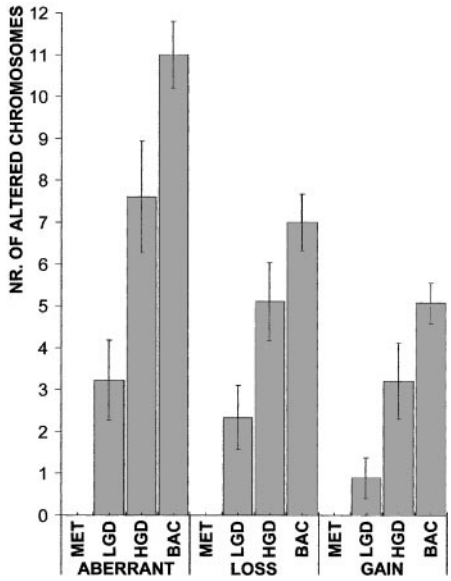

Fig. 2. Bar histogram showing the mean frequencies of the total number of aberrant chromosomes, chromosomes with loss, and chromosomes with gain in the subsequent stages of neoplastic progression, *i.e.*, metaplasia (*MET*), low-grade dysplasia (*LGD*), high-grade dysplasia (*HGD*), and Barrett's adenocarcinoma (*BAC*). *Error bars*, the SE. A gradual increase was found in the mean number of aberrant chromosomes, chromosomes with loss, and chromosomes with gain in the successive stages (Spearman's rank test; all $P < 0.001$; all r_s values ≥ 0.6).

No aberrations were detected in metaplasia by comparative genomic hybridization, which is in contrast with a recently published comparative genomic hybridization study on Barrett's esophagus (10). The latter study reported both losses and gains in metaplasias without dysplasia. We could not find alterations in metaplasias of both archival and fresh-frozen specimens. The absence of genomic aberrations in intestinal metaplasia without dysplasia is, to our opinion, in agreement with the low cancer risk reported for Barrett's esophagus (44). Moreover, loss of heterozygosity studies of metaplasia have been inconclusive in finding alterations. One report described lack of allelic imbalance in metaplasia at the *TP53*, *APC*, and *CDKN2A* gene loci (18), whereas another study detected allelic imbalance at the *APC* gene locus in metaplasia only if isolated adjacent to adenocarcinoma (25). A third report described allelic imbalance in metaplasia, but this might be due to the inclusion of "indefinite for dysplasia" specimens (16). However, as in our study, an increase in loss of heterozygosity was detected at the loci of *TP53*, *APC*, and *DCC* in the successive stages of metaplasia, low-grade dysplasia, high-grade dysplasia, and adenocarcinoma (16). Evaluation of the losses found in dysplasias on the long arm of chromosome 5 suggests that there might be two deleted regions. These two loci, 5q21-q22 and 5q31-q34, contain candidate genes *APC*, *MCC*, (5q21-q22) and *CTNNA1* (5q31), respectively. The latter codes for α-catenin, which is part of the E-cadherin-catenin complex. Finally, loss of 7q33-q35 might include *CASP2*, a gene involved in the regulation of apoptosis (45).

In conclusion, this study has revealed a variety of not only known, but also novel aberrations involved in malignant transformation of Barrett's esophagus, *e.g.*, high frequency loss on 7q, gain on 12p, and high-level amplifications on 7q and 17q in high-grade dysplasias. Some of the alterations might be useful biomarkers to discriminate between the different stages of neoplastic progression. Loss of 16q21-q22 and gain of 20q11.2-q13.1 were disclosed as potent discriminators between high-grade dysplasia and adenocarcinoma. Moreover, loss of 7q33-q35 appeared to be a novel marker to distinguish between low-grade dysplasia and high-grade dysplasia.

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