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# Comparative Genomic Hybridization of Cancer of the Gastroesophageal Junction: Deletion of 14Q31–32.1 Discriminates between Esophageal (Barrett's) and Gastric Cardia Adenocarcinomas[1]

**Herman van Dekken[2], Eric Geelen, Winand N. M. Dinjens, B. P. L. Wijnhoven, Hugo W. Tilanus, Hans J. Tanke, and Carla Rosenberg**

*Departments of Pathology [H. v. D., E. G., W. N. M. D., C. R.] and Surgery [B. P. L. W., H. W. T.], University Hospital Rotterdam, Erasmus University, 3000 DR Rotterdam, and The Rotterdam Esophageal Tumor Study Group and Department of Cytochemistry and Cytometry, State University Leiden, 2333 AL Leiden [E. G., H. J. T., C. R.], the Netherlands*

## ABSTRACT

**Incidence rates have risen rapidly for esophageal and gastric cardia adenocarcinomas. These cancers, arising at and around the gastroesophageal junction (GEJ), share a poor prognosis. In contrast, there is no consensus with respect to clinical staging resulting in possible adverse effects on treatment and survival. The goal of this study was to provide more insight into the genetic changes underlying esophageal and gastric cardia adenocarcinomas. We have used comparative genomic hybridization for a genetic analysis of 28 adenocarcinomas of the GEJ. Eleven tumors were localized in the distal esophagus and related to Barrett's esophagus, and 10 tumors were situated in the gastric cardia. The remaining seven tumors were located at the junction and could not be classified as either Barrett-related, or gastric cardia. We found alterations in all 28 neoplasms. Gains and losses were distinguished in comparable numbers. Frequent loss (≥25% of all tumors) was detected, in decreasing order of frequency, on 4pq (54%), 14q (46%), 18q (43%), 5q (36%), 16q (36%), 9p (29%), 17p (29%), and 21q (29%). Frequent gain (≥25% of all tumors) was observed, in decreasing order of frequency, on 20pq (86%), 8q (79%), 7p (61%), 13q (46%), 12q (39%), 15q (39%), 1q (36%), 3q (32%), 5p (32%), 6p (32%), 19q (32%), Xpq (32%), 17q (29%), and 18p (25%). Nearly all patients were male, and loss of chromosome Y was frequently noted (64%). Recurrent high-level amplifications (>10% of all tumors) were seen at 8q23–24.1, 15q25, 17q12–21, and 19q13.1. Minimal overlapping regions could be determined at multiple locations (candidate genes are in parentheses): minimal regions of overlap for deletions were assigned to 3p14 (*FHIT, RCA1*), 5q14-21 (*APC, MCC*), 9p21 (*MTS1/CDKN2*), 14q31–32.1 (*TSHR*), 16q23, 18q21 (*DCC, PI5*) and 21q21. Minimal overlapping amplified sites could be seen at 5p14 (*MLVI2*), 6p12–21.1 (*NRASL3*), 7p12 (*EGFR*), 8q23–24.1 (*MYC*), 12q21.1, 15q25 (*IGF1R*), 17q12–21 (*ERBB2/HER2-neu*), 19q13.1 (*TGFB1, BCL3, AKT2*), 20p12 (*PCNA*), 20q12–13 (*MYBL2, PTPN1*), and Xq25. The distribution of the imbalances revealed similar genetic patterns in the three GEJ tumor groups. However, loss of 14q31–32.1 occurred significantly more frequent in Barrett-related adenocarcinomas of the distal esophagus, than in gastric cardia cancers ($P = 0.02$). The unclassified, "pure junction" group displayed an intermediate position, suggesting that these may be in part gastric cardia tumors, whereas the others may be related to (short-segment) Barrett's esophagus. In conclusion, this study has, first, provided a detailed comparative genomic hybridization-map of GEJ adenocarcinomas documenting new genetic changes, as well as candidate genes involved. Second, genetic divergence was revealed in this poorly understood group of cancers.**

## INTRODUCTION

Analyses of cancer incidence data in the United States and Western Europe revealed steadily rising rates over the past decades of adenocarcinomas of the esophagus and gastric cardia (1, 2). Overall, cancer of the esophagus is increasing, and stomach cancer is decreasing. However, when analyzed by histological type and subsite the picture is very different. In the esophagus, squamous cell carcinoma rates have remained stable, whereas a rapid increase of adenocarcinoma is observed. In the stomach, cardia shows a very similar pattern to adenocarcinoma of the esophagus, but pyloric-antrum cancer is decreasing. Both esophageal and gastric cardia adenocarcinomas arise around the GEJ.[3] Esophageal adenocarcinoma is strongly correlated with Barrett's esophagus. In Barrett's esophagus, the squamous cell epithelium has undergone metaplastic change to columnar epithelium as a result of long-standing gastroesophageal reflux (3–5). Metaplastic change has also been observed at the GEJ, which might explain the rising frequency at this location (6). Intestinal metaplasia of the gastric cardia has been reported, however, its relation with malignant transformation is presently not clear (7).

GEJ adenocarcinomas share a poor prognosis, due to aggressive tumor behavior, as well as late detection (8, 9). Adenocarcinomas of the GEJ region disproportionately affect white men and less frequently occur among women (1, 10). A 5–6-fold increase has been reported between 1970 and 1990 (11, 12). The simultaneously increased incidence at the different locations suggest that adenocarcinomas of the GEJ are related. Reflux disease has been suggested as an etiological factor not only in esophageal adenocarcinoma, but also in cancer of the gastric cardia (13). Recent epidemiological studies have focused on the role of diet and cigarette smoking. It was found that smoking is a major risk factor for GEJ carcinomas (14). Also, increased fat intake was found to be important in esophageal and gastric cardia cancers (15, 16). In addition, an increasing prevalence of obesity may have contributed to the upward trends in GEJ adenocarcinomas (17).

Cytogenetic studies of series of both gastric and esophageal adenocarcinomas have shown frequent chromosomal rearrangement of 11p13–15 (18) and deletion of 3q (19). In a study of 37 adenocarcinomas in Barrett's esophagus and gastric cardia, loss of the Y chromosome seemed a prominent feature (20). Furthermore, rearrangements were most frequently seen of chromosome arms 1p, 3q, 11p, and 22q. Genetic abnormalities have been extensively documented in the, formerly common, pyloric-antrum type of gastric cancer (*e.g.*, gene amplification; Ref. 21). Ranzani *et al.* (22) detected LOH at 5q, 11p, 17p, and 18q and, with a low frequency, also at 7q and 13q. In these gastric cancers, deletions often occur at the *APC* and *MCC* loci on 5q21 (23). Little is known of LOH in

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[2] To whom requests for reprints should be addressed, at Department of Pathology, Erasmus University Rotterdam, P. O. Box 1738, 3000 DR Rotterdam, the Netherlands. Phone: 31-10-408-7901; Fax: 31-10-408-9487; E-mail: vandekken@path.fgg.eur.nl.

[3] The abbreviations used are: GEJ, gastroesophageal junction; CGH, comparative genomic hybridization; LOH, loss of heterozygosity; *MCC*, mutated in colorectal cancer; *APC*, adenomatosis polyposis coli; *MTS1*, multiple tumor suppressor 1; *DCC*, deleted in colorectal carcinoma; *FHIT*, fragile histidine triad gene; *MLV12*, MoMuLV integration site 2; *NRASL3*, v-*ras* neuroblastoma RAS-like oncogene 3; *EGFR*, epidermal growth factor receptor; *MYC*, v-*myc* myelocytomatosis oncogene; *ERBB2*, oncogene 2; *TGFβ1*, transforming growth factor β1; *BCL3*, B-cell CLL/lymphoma 3; *AKT2*, v-*akt* murine thymoma oncogene 2; *PCNA*, proliferating cell nuclear antigen; *MYBL2*, v-*myb* myeloblastosis-like oncogene 2; *PTPN1*, protein tyrosine phosphatase nonrec. type 1; *TSHR*, thyroid-stimulating hormone receptor; *IGF1R*, insulin-like growth factor 1 receptor; UICC, Union Internationale Contre le Cancer.

GEJ tumors. In a recent study of adenocarcinoma of the gastric cardia, frequent allelic loss was seen on 3p, 4q, 5q, 8p, 9pq, 12q, 13q, 17p, and 18q (24). Blount *et al.* (25) reported that alleles in chromosomes 5q, 17p, and 18q are involved in esophageal adenocarcinomas with high frequency. Allelotyping studies of esophageal adenocarcinoma have been constructed (26–28). They found frequent loss on 1p, 3q, 6q, 4pq, 5q, 9p, 12pq, 13q, 17p, and 18q. A few *in situ* hybridization studies have been reported on GEJ adenocarcinomas. In an early study, loss of chromosome Y and aneuploidy in gastric adenocarcinomas was shown (29). Hunter *et al.* (30) reported a high percentage of chromosome Y loss in esophageal adenocarcinoma. Krishnadath *et al.* (31, 32) described loss of chromosome Y, gain of chromosome 8, and loss of chromosome 17 in esophageal adenocarcinoma. Recently, CGH of GEJ cancers was reported on limited numbers of these neoplasms (33, 34). Gain of chromosome 20 was most frequently found. Furthermore, gain was seen on 6p, 7pq, 8q, and 17q, and loss was observed on 4pq, 5q, and 18q.

We undertook a CGH study to address the following questions: (*a*) what are the frequent unbalanced genetic abnormalities occurring in GEJ adenocarcinomas? (*b*) are there differences in the genetic profiles of Barrett-related esophageal adenocarcinomas *versus* gastric cardia cancers? and (*c*) is it possible to assign candidate genes underlying these neoplasms?

## MATERIALS AND METHODS

**Patient Material.** We collected 28 adenocarcinomas of the GEJ (*i.e.*, 11 esophageal, 10 gastric cardia, and 7 nonclassifiable cancers). The specimens were fresh-frozen, except four paraffin-embedded samples. The esophageal carcinomas were located in the distal esophagus in the presence of Barrett's transformed mucosa. The nonclassifiable tumors were situated at the GEJ, and Barrett's mucosa was not present. The gastric cardia cancers were clearly localized in the proximal stomach. The majority of the GEJ specimens were primary tumors ($n = 23$), but cell lines ($n = 3$; Ref. 35) and xenografts ($n = 2$) were included also (Table 1). Staging of the tumors was performed according to the UICC classification (36).

**CGH.** The 28 specimens were microdissected to enrich for tumor cell content (≥75% of cells). Excised tumor material was minced using a fine scalpel, and digested in extraction buffer [10 mM Tris/HCl (pH 8.0), 100 mM NaCl, 25 mM EDTA, 0.5% SDS, and 300 μg/ml Proteinase K] at 37°C for several hours. DNA was extracted with phenol-chloroform-isoamyl alcohol for at least four times and subsequently precipitated in ethanol, according to standard protocols. DNA was treated with RNase (20 μg/μl in 2 × SSC) for 1 h at 37°C, precipitated, and dissolved overnight in sterile water at 55°C. Concentration, purity, and molecular weight of the DNA was estimated using both UV spectrophotometry and ethidium bromide-stained agarose gels with control DNA series.

The CGH procedure was based on the protocol described by Kallioniemi *et al.* (37) with few modifications, as described previously by us (38). Tumor DNA was direct-labeled with FITC-dUTP, and control male DNA was labeled with lissamine-dUTP (both from DuPont-NEN, Boston, MA) by nick-translation. Each labeled DNA (200 ng) and 10 μg of human Cot-1 DNA (Life Technologies, Inc., Gaithersburg, MD), dissolved in 10 μl of hybridization buffer (50% formamide/2 × SSC/10% dextran sulfate), were hybridized to normal male metaphases and incubated at 37°C for 4 days. Posthybridization washes were performed with 2 × SSC at 37°C, followed by 0.1 × SSC at 60°C. Slides were counterstained with 4,6-diamidino-2-phenylindole (0.5 μg/ml) in Vectashield antifade solution (Vector Laboratories, Inc., Burlingame, CA).

Images of each of the three fluorochromes were collected using an epifluorescence microscope (Leica DM, Rijswijk, the Netherlands) equipped with three single excitation filters, a multibandpass dichroic mirror, a multiband emission filter (P-1 filter set; Chroma Technology, Brattleborough, VT), and a cooled CCD camera (Photometrics, Tucson, AZ). The green, red, and blue images were collected sequentially by changing the excitation filter. Images were saved using a routine built up in SCIL-Image (TNO, Delft, the Netherlands), implemented on a Power Macintosh 8100, and analyzed using QUIPS XL software (Vysis, Downers Grove, IL). For the profiles, losses of DNA sequences are defined as chromosomal regions where the mean green to red fluorescence ratio and its 95% confidence interval is below 0.9, whereas gains are defined as chromosomal regions where this ratio is above 1.1 (a 0.8–1.2 interval was used for the four paraffin-embedded samples). The threshold values were based on measurements from a series of normal controls.

Table 1 *Tumor/patient data*

| Tumor | Age/sex | Site | Type | Differentiation | Stage (UICC)[a] | Barrett |
|---|---|---|---|---|---|---|
| oe33 | 73/F | Esophagus | Cell line[b] | Poor | $T_{2/3}N_0M_0$ | Yes |
| bp1 | 69/M | Esophagus | Primary | Moderate | $T_3N_0M_0$ | Yes |
| bp2 | 49/M | Esophagus | Primary | Moderate | $T_3N_1M_0$ | Yes |
| bp7 | 61/M | Esophagus | Primary | Poor | $T_3N_1M_1$ | Yes |
| bp11 | 68/M | Esophagus | Primary | Poor | $T_3N_1M_1$ | Yes |
| ba3 | 53/M | Esophagus | Primary | Poor | $T_3N_0M_0$ | Yes |
| ba7 | 63/M | Esophagus | Primary | Moderate | $T_1N_0M_0$ | Yes |
| ba10 | 53/M | Esophagus | Primary | Moderate | $T_3N_1M_1$ | Yes |
| ba13 | 65/M | Esophagus | Primary | Poor | $T_3N_1M_0$ | Yes |
| ba22 | 73/M | Esophagus | Primary | Moderate | $T_3N_1M_1$ | Yes |
| ba27 | 85/M | Esophagus | Primary | | $T_3N_1M_1$ | Yes |
| oe19 | 72/M | Cardia | Cell line[b] | Moderate | $T_3N_1M_0$ | No |
| m2.1 | 76/M | Cardia | Xenograft[c] | Moderate | $T_2N_2M_0$ | No |
| ba1 | 63/M | Cardia | Primary | Well | $T_2N_1M_0$ | No |
| ba15 | 57/M | Cardia | Primary | Moderate | $T_2N_1M_0$ | No |
| ba18 | 54/M | Cardia | Primary | Poor | $T_3N_1M_0$ | No |
| ba23 | 75/M | Cardia | Primary | Moderate | $T_3N_1M_0$ | No |
| ba26 | 63/M | Cardia | Primary | Poor | $T_2N_0M_0$ | No |
| ba32 | 58/M | Cardia | Primary | Poor | $T_3N_2M_0$ | No |
| ba33 | 69/M | Cardia | Primary | Moderate | $T_2N_0M_0$ | No |
| ba34 | 75/F | Cardia | Primary | Moderate | $T_2N_2M_0$ | No |
| oe50 | 71/F | Junction | Cell line[b] | Poor | $T_{2/3}N_0M_0$ | No |
| m4.1 | 54/M | Junction | Xenograft[c] | Poor | $T_3N_2M_1$ | No |
| ba11 | 59/M | Junction | Primary | Poor | $T_3N_2M_0$ | No |
| ba17 | 78/M | Junction | Primary | Moderate | $T_3N_0M_0$ | No |
| ba19 | 67/M | Junction | Primary | Poor | $T_3N_2M_0$ | No |
| ba20 | 44/M | Junction | Primary | Poor | $T_3N_2M_0$ | No |
| ba21 | 62/M | Junction | Primary | Moderate | $T_3N_0M_0$ | No |

[a] Tumor-node-metastasis classification according to the UICC (36). Adenocarcinomas of the GEJ are classified as gastric cardia. It is important to note that in this classification distal oesophageal adenocarcinomas are relatively overrated in comparison with gastric cancers.
[b] Obtained from the European Collection of Animal Cell Cultures.
[c] Derived from a regional lymph node metastasis.

**Statistical Evaluation.** The two-tailed Fisher's exact test was used for comparison of genetic aberrations in the GEJ tumor groups.

## RESULTS

Our investigation concerned the CGH evaluation of 28 GEJ adenocarcinomas, (*i.e.*, 11 esophageal, 10 gastric cardia, and 7 nonclassifiable cancers of the junction; Table 1). The esophageal carcinomas were located in the distal esophagus in the presence of Barrett's transformed mucosa. The nonclassifiable tumors were situated at the gastro-esophageal junction, and Barrett's mucosa was not present. The gastric cardia cancers were clearly localized in the proximal stomach. We found multiple alterations in all 28 neoplasms, illustrating the genetic instability of gastrointestinal adenocarcinomas (Fig. 1). Gains and losses were seen in comparable numbers. Frequent loss (≥25% of all tumors) was detected, in decreasing order of frequency, on 4pq (54%), 14q (46%), 18q (43%), 5q (36%), 16q (36%), 9p (29%), 17p (29%), and 21q (29%). Frequent gain (≥25% of all tumors) was observed, in decreasing order of frequency, on 20pq (86%), 8q (79%), 7p (61%), 13q (46%), 12q (39%), 15q (39%), 1q (36%), 3q (32%), 5p (32%), 6p (32%), 19q (32%), Xpq (32%), 17q (29%), and 18p (25%). Examples are illustrated in Fig. 2. Nearly all patients were male, which is common in these cancers, and loss of chromosome Y was frequently noted (64%). Recurrent high-level amplifications (>10% of all tumors) were seen at 8q23–24.1, 15q25, 17q12–21, and 19q13.1. The 15q and 19q amplifications have not been reported in other human neoplasms before. Minimal overlapping regions could be determined at multiple locations (Table 2; candidate genes are in parentheses): minimal regions of overlap for losses were assigned to 5q14-21 (*APC, MCC*), 9p21 (*MTS1/CDKN2*), 14q31–32.1 (*TSHR*), 16q23, 18q21 (*DCC, PI5*), and 21q21. Less frequent (21% of the tumors), but clearly present, was a minimal deleted region on 3p14 (*FHIT, RCA1*). Minimal overlapping sites for gains could be seen at 5p14 (*MLVI2*), 6p12–21.1 (*NRASL3*), 7p12 (*EGFR*), 8q23–24.1 (*MYC*), 12q21.1, 15q25 (*IGF1R*), 17q12–21 (*ERBB2/HER2-neu*), 19q13.1 (*TGFB1, BCL3, AKT2*), 20p12 (*PCNA*), 20q12–13 (*MYBL2, PTPN1*), and Xq25. The genomic imbalances displayed similar distribution profiles in the three GEJ-tumor groups. However, loss of 14q31–32.1 occurred significantly more frequent in Barrett-related adenocarcinomas of the distal esophagus, than in gastric cardia cancers (Fisher's exact test, $P = 0.02$). The unclassified, pure junction group of tumors displayed an intermediate position (Fig. 3). It suggests that part of these may be gastric cardia tumors, whereas the others may be related to Barrett's transformed mucosa (*i.e.*, the so-called short-segment Barrett's esophagus).

## DISCUSSION

This study reports the first detailed CGH-map of genetic changes underlying adenocarcinomas of the GEJ. The overall genomic profile,

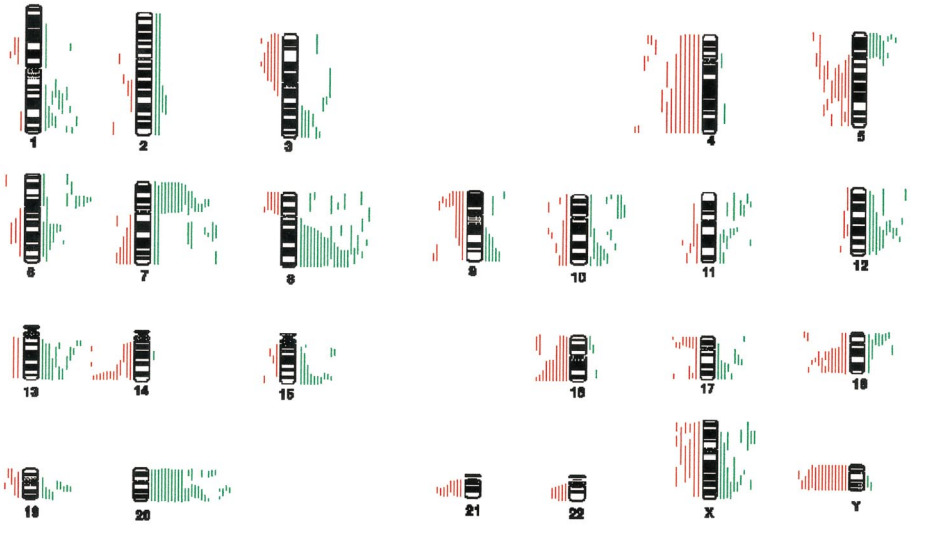

Fig. 1. Chromosomal ideograms showing the summary of DNA copy number changes, detected by CGH, in 28 adenocarcinomas of the GEJ (esophagus, 11; gastric cardia, 10; junction/nonclassifiable, 7). Losses are displayed on the left of the ideogram (*red*), gains are shown on the right (*green*). Frequent loss is detected on 4pq, 5q, 9p, 14q, 16q, 17p, 18q, 21q, and Y. Frequent gain is observed on 1q, 3q, 5p, 6p, 7p, 8q, 12q, 13q, 15q, 17q, 18p, 19q, 20pq, and Xpq.

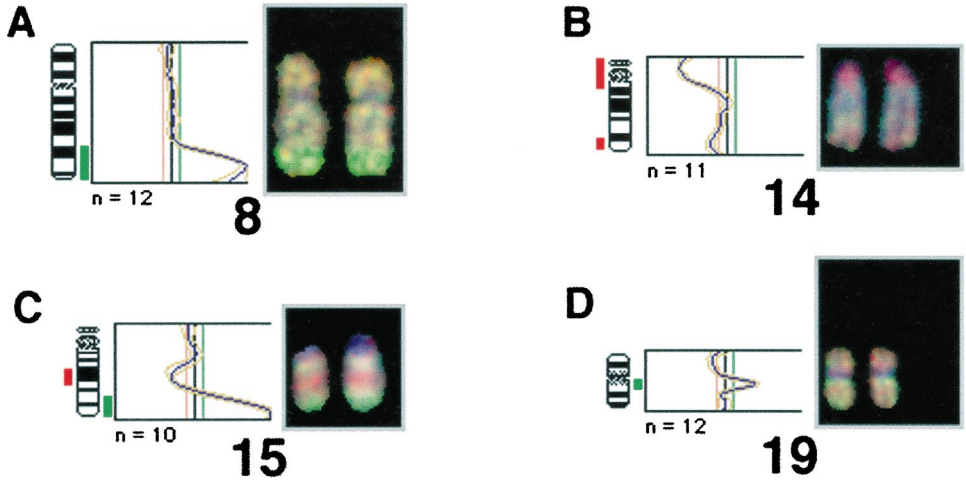


Fig. 2. CGH of four GEJ adenocarcinomas illustrating specific frequent deletions or high-level amplifications. The chromosomal ideograms are shown along with the ratio profiles and the digitized fluorescent images. *A*, high-level amplification of 8qter (MYC region) in an adenocarcinoma of the junction (ba11; nonclassifiable). *B*, deletion of 14q31–32.1 in an esophageal tumor cell line (oe33). *C*, amplification of 15qter, including 15q25, in a gastric cardia cancer (xenograft m2.1); also, a deletion is present, proximal from the amplification. *D*, high-level amplification of 19q13.1 (ba15; cardia carcinoma).

and the distribution of the chromosomal alterations, is clearly different from other frequently occurring human solid tumors, such as cancers of the breast, lung, or prostate (reviewed in Refs. 39 and 40). A number of new, not previously documented, genetic changes were detected (*e.g.*, loss on 14q and 16q, or gain of 5p and 13q). Other aberrations were confirmed, such as loss on 4pq, 5q, and 18q, or gain of 20pq and 8q. New high-level amplifications are reported (*i.e.*, on 15q25 and 19q13.1), and minimal regions were determined at several chromosomal sites. Furthermore, genetic divergence within GEJ adenocarcinomas was disclosed. Below, we will discuss the most prevalent and/or relevant alterations, as well as candidate genes (Tumor suppressor and oncogenes described below are reviewed in Refs. 41–43).

**Chromosomal Losses.** The most frequent loss seemed to involve chromosome 4, the long arm slightly more than the short arm. This is in agreement with previous molecular studies in which frequent LOH on 4q was found (27). So, far, no genes have been implicated for the deletion in this region.

About 50% of the neoplasms exhibited loss on 14q with a minimal region at 14q31–32.1. This region is clearly different from the 14q loss that is frequently encountered in gastrointestinal stromal tumors (*i.e.*, 14q22; Ref. 44). A possible candidate gene residing in our chromosomal region is the *TSHR*, a gene involved in adenocarcinomas of the thyroid gland. Interestingly, this deletion points at genetic divergence in GEJ cancers: Frequent loss (64%) was seen in Barrett-related esophageal adenocarcinomas, but not in gastric cardia tumors (10%; $P = 0.02$). This might be associated with different cancer stem cells in Barrett's transformed esophageal mucosa, a condition not known in the proximal stomach. The "unclassified" group displayed an intermediate status (43% loss), which is possibly related to the presence of a so-called short-segment Barrett's esophagus (6).

The loss encountered on 3p14 (21% of the tumors) seems to involve the *FHIT* gene. Recently, frequent deletions of *FHIT* were reported in Barrett's esophagus and adenocarcinoma (45). Some of the deleted areas could be connected with known tumor suppressor genes, such as *MCC* (5q21), *MTS1* (9p21), or *DCC* (18q21), whereas other regions might harbor yet unknown suppressor genes (*i.e.*, 16q23 and 21q21). We found loss on 17p in ~30% of the cancers. LOH of the p53 region has been reported in varying frequencies in esophageal adenocarcinoma (26, 27).

Table 2 *List of genes, potentially involved in frequent alterations (amp,[a] del) of adenocarcinomas of the GEJ*

| CGH aberration | Candidate gene | Location | Function |
|---|---|---|---|
| 3p14 del | *FHIT* | 3p14.2 | Suppression |
| | *RCA1* | 3p14.2 | Suppression |
| 5p14 amp | *MLV12* | 5p14 | Activation (?) |
| 5q14-21 del | *MCC* | 5q21 | Suppression |
| | *APC* | 5q21-22 | Suppression |
| 6p12-21.1 amp | *NRASL3* | 6p12-ter | Activation |
| 7p12 amp | *EGFR* | 7p12 | Activation |
| 8q23-24.1 amp | *MYC* | 8q24.1 | Activation |
| 9p21 del | *MTS1* | 9p21 | Suppression |
| 14q31-32.1 del | *TSHR* | 14q31 | Suppression |
| 15q25 amp | *IGF1R* | 15q25-26 | Activation (?) |
| 17q12-21 amp | HER2-neu | 17q12-21 | Activation |
| | *ERBB2* | | |
| 18q21 del | *DCC* | 18q21.3 | Suppression |
| | PI5 | 18q21.3 | Suppression |
| 19q13.1 amp | *TGFβ1* | 19q13 | Activation |
| | *BCL3* | 19q13 | Activation |
| | *AKT2* | 19q13 | Activation |
| 20p12 amp | *PCNA* | 20p12 | Activation (?) |
| 20q12-13 amp | *MYBL2* | 20q13.1 | Activation |
| | *PTPN1* | 20q13 | Activation (?) |

[a] amp, gain/amplification; del, deletion; *RCA1*, renal carcinoma, familial-associated 1;*HER2-neu*, V-erb-b2; *PI5*, protease inhibitor 5 (maspin).

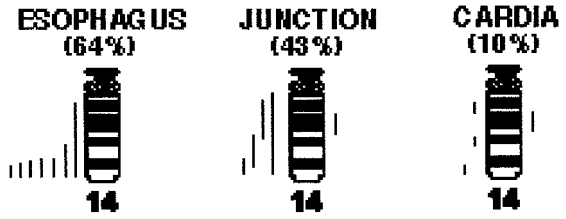


Fig. 3. Chromosome 14 ideograms demonstrating the prevalence of distal 14q deletions (14q31–32.1) in the three GEJ groups. Note frequent loss in Barrett-related esophageal adenocarcinomas, in contrast to gastric cardia tumors ($P = 0.02$). The unclassified junction tumors display an intermediate position.

**Chromosomal Gains.** The most frequent gain occurred at chromosome 20 (86% of all tumors), the long arm (20q12–13) being slightly more altered than the short arm (20p12). The latter gain might involve *PCNA*. Loss of 20q has been reported in various solid cancers and was found associated with a poor prognosis in breast cancer (46). Several candidate genes have been suggested, among them *MYBL2* or *PTPN1*, a nonreceptor tyrosine phosphatase involved in growth regulation (47).

Numerous gain (79%) was also seen of the long arm of chromosome 8 (see also "Amplifications"). Gain of 8q, often involving the whole chromosome arm, is seen in many human cancers (*e.g.*, prostate cancer; Ref. 40). Other chromosomal areas, frequently gained, were 5p14, 6p12–21.1, 7p12, 12q21.1, and Xq25. The gain of 7p12 is likely to be associated with the *EGFR*, a gene often involved in human neoplasms. The gain of 5p14 might implicate a leukemia virus integration site (*MLVI2*), and 6p gain is possibly related to *NRASL3*, which belongs to the *RAS* superfamily (47).

**Amplifications.** A distinction is made between gains and amplifications, as pointed out recently by Knuutila *et al.* (47). True amplifications, likely representing chromosomal amplicons, are seen as a distinct and high peak in the CGH profile of a given neoplasm. However, there is a gray area between gain and amplification, which is clearly illustrated in our series by the 7p12 region (*EGFR*). This site showed frequent and specific gain, but no high-level amplification. We observed amplifications (>10% of all tumors) for the following chromosomal regions: 8q23–24.1, 15q25, 17q12–21, and 19q13.1. The 8q and 17q amplicons are likely to be associated with, respectively, *MYC* and *ERBB2*, oncogenes frequently involved in various cancers. However, the amplifications at 15q and 19q, possibly characteristic for GEJ adenocarcinomas, are not easily attributed to a candidate gene because they have not been described before as putative oncogenic sites.

The 15qter amplification might implicate the *IGF1R* gene, which has been reported to be sporadically amplified in human breast cancer, in some cases with coamplification of *FES*, residing at 15q26.1. (48). The amplification at 19q31.1 could be associated with *TGFβ*, *BCL3*, or *AKT2*, a murine thymoma oncogene homologue (Table 2). However, further investigation is needed to elucidate the nature of the chromosome 15 and 19 amplifications, which might play an important role in GEJ cancers.

In conclusion, this study has revealed a variety of chromosomal aberrations in GEJ cancers. Some of these changes might be found in all types of cancer, whereas others might be associated with epithelial neoplasms. The genomic patterns of the three GEJ tumor types, revealed by CGH, are markedly similar, indicating that they are a closely related entity. However, one genetic change (del14q) was distinguished that varied between the three groups, suggesting some degree of tissue specificity. Presently, we are further investigating this region by LOH analysis.[4] Overall, the CGH profile of

[4] B. P. L. Wijnhoven, unpublished data.

GEJ cancer is clearly different from other malignancies. Therefore, the deletion of 14q31–32.1 or the amplifications at 15q25 and 19q13.1, might have diagnostic use for adenocarcinomas of the GEJ.

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