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# DNA Sequence Copy Number Changes in Gastrointestinal Stromal Tumors: Tumor Progression and Prognostic Significance[1]

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## ABSTRACT

**To identify genetic changes related to tumor progression and find out diagnostic and prognostic genetic markers in gastrointestinal stromal tumors (GISTs), 95 tumor samples (24 benign GISTs, 36 malignant primary GISTs, and 35 GIST-metastases) from 60 patients were studied using comparative genomic hybridization. DNA copy number changes were detected in all samples. Benign GISTs had a mean of 2.6 aberrations/sample (losses:gains, 5:1) and significantly fewer DNA copy number changes and fewer gains than malignant primary and metastatic GISTs ($P < 0.01$). High-level amplifications were not seen in benign GISTs. Malignant primary GISTs had a mean of 7.5 aberrations/tumor (losses: gains, 1.6:1), whereas the mean number of aberrations/metastatic GIST was 9 (losses:gains, 1.8:1). Frequent changes observed in all GIST groups included losses in chromosome arms 1p (51%), 14q (74%), and 22q (53%). Gains and high-level amplifications at 8q and 17q were significantly more frequent in metastatic GISTs (57 and 43%) than in benign GISTs (8 and 0%; $P < 0.001$) and malignant primary GISTs (33 and 25%; $P < 0.05$). Gains and high-level amplifications at 20q were only seen in malignant primary and metastatic GISTs ($P < 0.01$), and gains at 5p were not detected in benign GISTs ($P < 0.01$). Losses in chromosome arm 9p were never seen in benign tumors ($P < 0.001$), and they were more frequent in metastatic GISTs than in malignant primary GISTs (63 and 36%; $P < 0.05$). Losses in 13q were less frequent in benign GISTs than in malignant primary ($P < 0.05$) and metastatic ($P < 0.01$) GISTs. Our results show that several DNA copy number changes are related to the behavior of GISTs and can be used as prognostic markers for tumor progression.**

## INTRODUCTION

GISTs[3], classified previously as smooth muscle tumors, constitute the most important group of primary mesenchymal tumors of the gastrointestinal tract. These tumors occur at all levels of the gastrointestinal tract and usually present between the sixth and eighth decades. Immunohistochemically, GISTs are usually positive for KIT (CD117) and CD34, variably positive for smooth muscle actin, and usually negative for desmin, in contrast to true smooth muscle tumors. GISTs are negative for S100 protein, in contrast to schwannomas (1, 2).

Clinically and pathologically, GISTs represent a spectrum of tumors including benign and malignant variants, the latter of which are generally identified by mitotic activity (>1 mitosis/10 high-power field). In some cases, the prediction of biological potential is difficult, because larger tumors with lower mitotic activity may also occasionally metastasize.

CGH enables screening of entire tumor genomes for gains and losses of DNA copy number and consequent mapping of aberrations to chromosomal subregions (reviewed in Refs. 3 and 4). Recently, we reported using CGH that loss of genetic material at chromosome arm 14q is the most frequently occurring aberration in both benign and malignant primary GISTs (5). The DNA copy number changes seen in GISTs were not detected in leiomyomas and leiomyosarcomas (6). Therefore, the immunophenotypic characteristics and the genetic profile of GISTs have clearly placed it as a separate tumor entity different from other mesenchymal tumors of the gastrointestinal tract. However, the prognostic evaluation of GISTs remained a difficult issue, requiring a complex multiparametric approach.

This study was performed to analyze the CGH changes in benign and malignant primary GISTs and to investigate whether progressive DNA copy number changes occur in recurrent and metastatic GISTs. We also investigated whether any DNA copy number change has prognostic significance for GISTs.

## MATERIALS AND METHODS

**Tumors.** Ninety-five GIST samples from 60 patients were included in the study. The samples consisted of 24 benign GISTs, 36 malignant primary GISTs, and 35 GIST metastases that were available from 15 of the malignant primary GISTs. Immunohistochemically identified leiomyomas and leiomyosarcomas (desmin-positive, CD117-negative) and schwannomas (S100-protein positive, CD117-negative) were excluded from the study. Most GISTs were CD117 and CD34 positive (92 and 72%, respectively). The categorization of GISTs into benign and malignant tumors was based on mitotic counts. Benign GISTs had <2 mitoses/10 high-power field. Malignant or potentially malignant GISTs had >1 mitosis/10 high-power fields. Follow-up data for 6–209 months (mean, 41 months) were available for all patients except four (nos. 25, 26, 29, and 48). None of the patients with benign tumors showed recurrence or metastases.

**CGH.** Because CGH sensitivity requires at least 50% of tumor material within a sample, paraffin-embedded tissue sections were dissected to obtain at least 70% of tumor cells. DNA from paraffin-embedded tissue sections was extracted as described earlier (7).

CGH was performed according to standard procedures with a modification using a mixture of fluorochromes conjugated to dCTP and dUTP nucleotides for nick translation (8). Hybridizations, washings, and ISIS digital image analysis (Metasystems GmbH, Altlussheim, Germany) were performed as described elsewhere (5).

**Controls.** In each CGH experiment, a negative control (peripheral blood DNA from a healthy donor) and a positive control were included. The positive control was a gastric tumor with known DNA copy number changes. On the basis of our earlier reports and the control results, we used 1.17 and 0.85 as cutoff levels for gains and losses, respectively. All of the CGH results were confirmed using a 99% confidence interval.

**Statistical Analysis.** All of the CGH results were confirmed using a 99% confidence interval. Briefly, intra-experiment SDs for all positions in the CGH ratio profiles were calculated from the variation of the ratio values of all homologous chromosomes within the experiment. Confidence intervals for the ratio profiles were then computed by combining them with an empirical inter-experiment SD and by estimating error probabilities based on the *t* distribution. For the analysis of the frequencies of DNA copy number changes in primary and metastatic GISTs, we used Fisher's exact two-tailed test. *P*s <0.05 were considered significant.

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[3] The abbreviations used are: GIST, gastrointestinal stromal tumor; CGH, comparative genomic hybridization.

Table 1 *Clinical, histological, and CGH karyotype findings of 95 gastrointestinal stromal tumors obtained from 60 patients*

Metastases from the same patient have the same number as the primary tumor but with a different letter, e.g. b, c, d.

| No. | Age/sex/ code | Histology | Site[a] | Size[b] | Follow-up in months | CGH karyotype[a] | |
|---|---|---|---|---|---|---|---|
| | | | | | | Losses | Gains and high-level amplifications |
| 1 | 59/m G1 | Benign | Jejunum | 5, 5 | NED, 51 | 1p, 15, 18, 22 | |
| 2 | 45/m G2 | Benign | Stomach | 4, 5 | NED, 45 | 14 | |
| 3 | 50/m G12 | Benign | Stomach | 3, 3 | NED, 38 | 1p, 14 | 8q |
| 4 | 75/m G25 | Benign | Stomach | 3 | NED, 87 | 14, 22 | |
| 5 | 63/m G9 | Benign | Stomach | 3, 5 | NED, 41 | 14q11–q13, 14q23–qter, 22 | |
| 6 | 61/f G10 | Benign | Stomach | 7 | NED, 40 | 1p, 14 | |
| 7 | 81/m G13 | Benign | Stomach | 5, 5 | NED, 110 | 15 | |
| 8 | 80/m G14 | Benign | Stomach | 5 | NED, 124 | 14 | |
| 9 | 9/73/f G18 | Benign | Stomach | 3, 5 | NED, 81 | 11p, 14 | 5q22–qter |
| 10 | 63/f G19 | Benign | Jejunum | 2 | NED, 31 | 1p, 11, 14, 15, 21 | 1q, 5, 7p, 18q |
| 11 | 58/m G21 | Benign | Stomach | 4 | NED, 210 | 14q13–qter, 22 | |
| 12 | 49/f G23 | Benign | Stomach | 16 | NED, 209 | 22q12–qter | |
| 13 | 65/f G24 | Benign | Stomach | 3 | NED, 28 | 14, Xq | |
| 14 | 73/m G26 | Benign | Stomach | 2 | NED, 82 | 14, 15, 21, 22 | |
| 15 | 79/f G29 | Benign | Stomach | 2 | NED, 133 | 22q12–qter | |
| 16 | 78/m G32 | Benign | Stomach | 2, 5 | NED, 75 | 14, 22 | |
| 17 | 80/m G35 | Benign | Jejunum | 4, 5 | NED, 24 | 1p, 6q, 13q21–qter, 14 | 19 |
| 18 | 85/m G38 | Benign | Stomach | 5 | NED, 21 | 14, 22 | |
| 19 | 69/m G41 | Benign | Jejunum | 2 | NED, 14 | 1p, 11p, 13, 14q11–q12, 15, 22 | |
| 20 | 67/f G44 | Benign | Stomach | 9 | NED, 6 | 14 | |
| 21 | 53/m G48 | Benign | Stomach | 6 | NED, 31 | 14 | |
| 22 | 34/m G49 | Benign | Stomach | 6, 5 | NED, 32 | | 7, 8, 11 |
| 23 | 41/m G52 | Benign | Jejunum | 7 | NED, 13 | 1p31.2–pter, 6q, 22 | |
| 24 | 68/m G60 | Benign | Stomach | 2 | NED, 33 | 14 | |
| 25 | 74/f G15 | Malignant | Stomach | NA | NA | 14q13–qter, 15q15–qter, 18 | 16, 17 |
| 26 | 60/f G22 | Malignant | Stomach | 4 | NA | 10q | 3q, **5**, 7, 12, 13, 18 |
| 27 | 59/m G47 | Malignant | Colon | 18 | NED, 12 | 1p, 6q, 8p22–pter, 10, 11q14–qter, 12, 14, 15, 18q11–q23 | 2q22–qter |
| 28 | 52/m G6 | Malignant | Stomach | 15 | DOD, 5 | 3cen–p21, 14 | 1q32–qter, 3p22–pter, 3q **(3q26–q29)** 8p12–qter |
| 29 | 72/m G17 | Malignant | Jejunum | 15 | NA | 2q31–qter, 3p, 9p | |
| 30 | 60/m G20 | Malignant | Jejunum | 10 | DOD, 9 | 1p, 13, 14cen–q23, 15, 21, 22 | **3q,** 8, **16p,** 17 |
| 31 | 68/f G27 | Malignant | Stomach | 15 | DOD, 71 | 1cen–p31, 3p, 14q21–q23, 15q21–qter | 2q34–q37 |
| 32 | 88/f G31 | Malignant | Stomach | 6 | DOD, 26 | 1cen–p31, 8p, 9q, 10p15–q22, 22, X | 1p32–pter, 5p, 9p, 17q21–q25 |
| 33 | 63/m G36 | Malignant | Colon | 16 | NED, 24 | 8p21–pter, 10pter–q22, 14q21–q32 | 1p, 5, 7p, 8q22–qter, 9q, 19, 20 |
| 34 | 63/m G11 | Malignant | Rectum | 7 | NED, 39 | 8p, 9, 13q21–qter, 14, 15q15–q22, 22 | 3, 5, **8q,** 11, 12, 17, 18, X |
| 35 | 86/f G16 | Malignant | Small intestine | 6, 5 | DOD, 19 | 1p, 2p, 15 | |
| 36 | 43/f G28 | Malignant | Jejunum | 8 | NED, 78 | 1cen–p31, 13q21–qter, 15q15–qter, 18q | 4, 17, 18p, 19 |
| 37 | 69/m G30 | Malignant | Stomach | 11 | DOD, 32 | | 11q14–q22, 19, X |
| 38 | 41/m G42 | Malignant | Stomach | 18 | NED, 12 | 1p, 2p13–pter, 9p, 14, 15, 22 | 11q |
| 39 | 44/m G43 | Malignant | Stomach | 17 | NED, 9 | 22 | 5, 7 |
| 40 | 73/f G45 | Malignant | Stomach | 4 | NED, 8 | | **5,** 6p, 7 |
| 41 | 71/f G46 | Malignant | Jejunum | 9 | NED, 27 | 1p, 4, 10, 15, 22 | |
| 42 | 73/f G50 | Malignant | Jejunum | 9 | Alive, 9 | 1p, 2q, 9p, 13, 14, 15, 18q, 22 | 5p14–pter |
| 43 | 59/m G58 | Malignant | Stomach | 12 | NED, 27 | 9, 14 | 4p, 5, 7 |
| 44 | 37/f PR28 | Malignant | Small intestine | NA | DOD, 50 | 1p, 8p, 9p, 11p, 14, 18q, 22 | 1q11–q31, 8q, 9q, 16q, 19p, 20q |
| 45 | 73/f G65 | Malignant | Duodenum | 6, 5 | NED, 24 | 1p, 6q21–qter, 9p, 11, 13, 14, 15 | 7 |
| 46 | 46/f K1 | Malignant | Stomach | 10 | Alive, 35 | 7p15–p22, 7q32–qter, 9p21.2–pter, 14, 15, 18p, 22q13 | 2q, 4q, 8q23–qter |
| 46b | K2 | Metastases | Omentum | | | 7p15–p22, 7q32–qter, 9p21.2–pter, 13q13–qter, 14, 15, 19q13.2–qter, 22q13 | 2q, 6p, 8q23–qter, 17q |
| 46c | K3 | Metastases | Colon mesentry | | | 7p15–pter, 7q32–qter, 9p21.2–pter, 14, 15, 19q13.2–qter, 22q13 | 2q, 4q, **8q23–qter, 17q** |
| 46d | K4 | Metastases | Omentum | | | 4p, 7p15–p22, 7q32–qter, 9p21.2–pter, 10, 11, 13q13–qter, 14, 15, 19q13.2–qter, 22q13 | **8q23–qter, 17q** |
| 46e | K5 | Metastases | Liver | | | 4p, 7p15–p22, 7q32–qter, 9p21.2–pter, 11p, 13q13–qter, 14, 15, 19q13.2–qter, 22q13 | 2q, **8q23–qter, 17q** |
| 46f | K6 | Metastases | Liver | | | 1p34.3–pter, 4, 6q14–qter, 7p15–p22, 7q32–qter, 9, 14, 18, 22q13 | 1q, 5 |
| 46g | K7 | Metastases | Colon mesentry | | | 7p15–p22, 7q32–qter, 9p21.2–pter, 14, 15, 18p, 19q13.2–qter, 22q13 | **2q,** 4q, **8q23–qter, 17q** |
| 46h | K8 | Metastases | Colon mesentry | | | 7p15–p22, 7q32–qter, 9p21.2–pter, 13q13–qter, 14, 15, 19q13.2–qter, 22q13 | 2pter–q13, 4q, 8q22–qter, 17q |
| 46l | K9 | Metastases | Colon mesentry | | | 1p, 7p15–p22, 7q32–qter, 9p21.2–pter, 11, 13q, 14, 15, 19q13.2–qter, 22q13, X | 2q, 4q, 8q22–qter **(8q23–qter), 17q** |
| 46j | K10 | Metastases | Colon mesentry | | | 7p15–p22, 7q32–qter, 9p21.2–pter, 13q13–qter, 14, 15, 19q13.2–qter, 22q13 | 2pter–q13, 4q, 8q22–qter, 17q |
| 46k | K11 | Metastases | Ovary | | | 7p15–p22, 7q32–qter, 9p21.2–pter, 11, 13, 14, 15, 19q13.2–qter, 22q13, X | 2q, 4q, **8q22–qter, 17q** |
| 46l | K12 | Metastases | Colon mesentry | | | 7p15–p22, 7q32–qter, 9p21.2–pter, 13q13–qter, 14, 15, 19q13.2–qter, 22q13 | 2pter–q13, 8q23–qter, 17q |
| 46m | K14 | Metastases | Colon mesentry | | | 6q14–qter, 7p11–p15, 7q32–qter, 9pter–q34.1, 14, X | |
| 47 | 67/m PR1 | Malignant | Jejunum | 7 | DOD, 35 | 1p, 2, 11, 13, 14, 15, 18 | |
| 47b | PR2 | Metastases | Abdomen | | | 1p, 2, 11, 13, 14, 15 | 1q, 3, 5, 6p, 7, 8, 9 |
| 47c | PR48 | Metastases | Abdomen | | | 1p, 2, 11, 13, 14, 15, 18 | 1q11–q24, 3p14–p22, 6p, 7, 8 |
| 47d | PR3 | Metastases | Abdomen | | | 1p, 2p22–pter, 11, 13, 14, 15, 18 | 1q, 3, 6p, 7, **8,** 9 |
| 48 | 44/m G8 | Malignant | Stomach | 17 | NA | 1q32–qter, 14 | 8q22–qter |
| 48b | PR5 | Metastases | Abdomen | | | 1p, 2q, 5p14.3–pter, 12q15–qter, 14, 19q | 8 |
| 49 | 64/m PR6 | Malignant | Small intestine | 12 | Alive, 24 | 1p, 6q, 13, 14, 15, 18, 22 | 5p, 6p, 7, 12q |
| 49b | PR7 | Metastases | Liver | | | 1p, 6q, 13, 14, 15, 18, 22 | 5p, 6p, 7, 12q |
| 50 | 59/f PR8 | Malignant | Stomach | 15 | Alive, 96 | 9pter–q31, 10p, 13, 14, 19q13.3–qter, X | 2p14–pter, 4p15–pter, 6p, 8, 9q32–qter, 10q, 12, 17q, 19pter–q13.2, 20q |
| 50b | PR9 | Metastases | Liver | | | 9pter–q31, 10p, 13, 14, 19q13.3–qter, X | 2p14–pter, 7, 8, 12, 17, 19pter–q13.2, 20q |

Table 1 *Continued.*

| No. | Age/sex/ code | Histology | Site[a] | Size[b] | Follow-up in months | CGH karyotype[a] | |
|---|---|---|---|---|---|---|---|
| | | | | | | Losses | Gains and high-level amplifications |
| 51 | 54/m PR10 | Malignant | Jejunum | 15 | Alive, 22 | 1p, 2, 7p, 11, 15, 18, 22 | 4q, 7q, 9p, 12q |
| 51b | PR11 | Metastases | Abdomen | | | 1p, 2, 4p, 11, 12p, 13, 15, 17p, 18, 22 | 5q, 7q, 8q, **9p,** 21 |
| 51c | PR12 | Metastases | Abdomen | | | 1p, 2p, 13, 15, 18, 22 | 5q, 10p |
| 52 | 59/m PR13 | Malignant | Small intestine | 20 | DOD, 49 | 1p, 8, 13, 14, 15, 22 | 1q, 2q22–qter, 4p, 5p, 17, 20p |
| 52b | PR14 | Metastases | Abdomen | | | 1p, 3pter–q13, 4q, 8, 9pter–q21, 10, 13, 14, 15, 22 | 1q, 2q22–qter, 4p, 5p, 17, 20p |
| 52c | PR15 | Metastases | Abdomen | | | 1p, 3pter–q13, 4q, 8, 9pter–q21, 13, 14, 15, 22 | 1q, 2q22–qter, 5p, 17, 20p |
| 53 | 62/f PR16 | Malignant | Stomach | 15 | Alive, 60 | 6, 9, 12pter–q22, 14, 22 | 17 |
| 53b | PR17G5 | Metastases | Abdomen | | Alive | 9, 14, 22 | 17q |
| 53c | PR18 | Metastases | Abdomen | | | 9, 14, 22 | 4, 17q, 20 |
| 54 | 59/m PR19G3 | Malignant | Ileum | 12 | DOD, 14 | 13, 14q11–q12 | 5p |
| 54b | PR20 | Metastases | Liver | | | 12p, 14 | 5p, 8q |
| 55 | 38/f PR21 | Malignant | Jejunum | 10 | DOD, 59 | 1p, 11q14–qter, 13, 14, 15, 18, 22, X | 1q, 5p, 8 |
| 55b | PR22 | Metastases | Abdomen | | | 1p, 15, 18, 22q13 | 1q, X |
| 55c | PR23 | Metastases | Abdomen | | | 1p, 15, 18, 22q13 | 1q |
| 55d | PR24 | Metastases | Abdomen | | | 1p, 13, 15, 18, 22 | 1q |
| 56 | 52/f PR33 | Malignant | Small intestine | 10 | DOD, 12 | 1p, 14, 15 | 7, 8, 12q22–qter |
| 56b | PR34 | Metastases | Abdomen | | | 1p, 14, 15 | 7, 8, 12q22–qter |
| 57c | PR35 | Metastases | Abdomen | | | 1p, 14, 15 | 7, 8, 12q22–qter |
| 58 | 61/m PR36 | Malignant | Stomach | 25 | DOD, 19 | 1p, 11, 13, 14, 15 | 2q, 8q23–qter, 17q, X |
| 58b | PR37 | Metastases | Abdomen | | | 1p, 9 | 20 |
| 58c | PR38 | Metastases | Abdomen | | | 1p, 9 | 17q22–qter, 20 |
| 59 | 39/m PR39 | Malignant | Stomach | 20 | DOD, 14 | 4q27–q34, 9, 14, 22 | |
| 59b | PR40 | Metastases | Abdomen | | | 4q27–q34, 9, 14, 22 | |
| 59c | 33/m PR42 | Metastases | Abdomen | | | 1p11–p32, 4, 9pter–q33, 14 | 20q13.2–qter |
| 60 | 41/f PR45 | Malignant | Stomach | 6 | Alive, 6 | 1p35–pter, 4, 9, 15, 22 | 5, 8p12–pter, 8q23–qter, 12q14–qter, 13, 18q |
| 60b | PR46 | Metastases | Abdomen | | | 1p35–pter, 4, 6q, 9, 14q11–q21, 15, 22 | 5, 8p12–pter, 8q23–qter, 12 **(12q22–qter),** 13, 18q, 20q |
| 60c | PR47 | Metastases | Abdomen | | | 1p35–pter, 4, 9, 10, 14, 15, 22 | 5p, 5q32–qter, 8p12–pter, 8q23–qter, 12 **(12q22–qter),** 13, 20q |

[a] All metastases were intra-abdominal.
[b] Maximum size in cm, high.
[c] High-level amplifications are in bold; NED, no evidence of disease; NA, not available; DOD, died of disease.

## RESULTS

Changes in DNA copy numbers were detected in all samples. DNA copy number changes were seen in all chromosomes in malignant primary and metastatic GISTs, whereas fewer chromosomes were involved in benign GISTs (Figs. 1 and 2). Benign GISTs had a mean of 2.6 aberrations/case (losses:gains, 5:1) and contained significantly fewer DNA copy number changes and fewer gains than malignant primary and metastatic GISTs ($P < 0.01$). High-level amplifications were not seen in benign GISTs. In malignant primary GISTs, the mean number of aberrations/case was 7.5 (losses:gains, 1.6:1), whereas the mean was 9 in metastatic GISTs (losses:gains, 1.8:1). The CGH profiles in metastatic GISTs and malignant primary tumors were similar, but metastatic GISTs showed a larger number of high-level amplifications. There was no correlation between the location and any specific DNA copy number changes.

Table 2 *Summary of significant DNA copy number changes in GISTs*

DNA copy number changes seen in primary malignant and metastatic tumors were compared to benign lesions. *P*s are shown in parentheses. *P*s shown in italics indicate comparison between metastatic and primary malignant tumors. Gains are indicated with + and losses with −.

| Chromosomal region | Benign GIST | Malignant GIST | Metastatic GIST |
|---|---|---|---|
| 8q+ | 8% | 33% (<0.001) | 57% (<0.001), *(<0.05)* |
| 17q+ | 0 | 25% (<0.05) | 43% (<0.001), *(<0.05)* |
| 20q+ | 0 | 11% (<0.001) | 26% (<0.001), *(<0.05)* |
| 5p+ | 0 | 31% (<0.001) | 29% (<0.001) |
| 9p− | 0 | 36% (<0.001) | 63% (<0.001), *(<0.05)* |
| 13q− | 8% | 36% (<0.05) | 46% (<0.001) |

Changes that were frequently detected in all GISTs with no statistical differences between benign, malignant primary, and metastatic tumors included losses in chromosome arms 1p (51%), 14q (74%), and 22q (53%).

Gains and high-level amplifications at 8q and 17q were significantly more often detected in metastatic GISTs (57 and 43%) than in benign GISTs (8 and 0%; $P < 0.001$) and malignant primary GISTs (33 and 25%; $P < 0.05$). Gains and high-level amplifications at 20q were only seen in malignant primary and metastatic GISTs ($P < 0.01$), and gains at 5p were not detected in benign GISTs ($P < 0.01$). Losses in 9p were never seen in benign tumors ($P < 0.001$), and they were more frequent in metastatic GISTs than in malignant primary tumors (63 and 36%; $P < 0.05$). The losses in 13q were less frequent in benign GISTs than in malignant primary ($P < 0.05$) and metastatic ($P < 0.01$) GISTs. In addition, several other changes were seen more frequently in malignant primary and metastatic GISTs than in benign GISTs. The details of DNA copy number changes are shown in Table 1 and Fig. 1. Fig. 2 shows the relative frequencies of the aberrations in all GISTs, and Table 2 summarizes the significant DNA copy number changes.

## DISCUSSION

On the basis of a large series of clinically benign and malignant GISTs and their metastases, we investigated the possible value of CGH to identify genetic markers of tumor behavior. The observation that benign tumors, almost exclusively, contain losses rather than gains, whereas malignant primary GISTs and their metastases contain

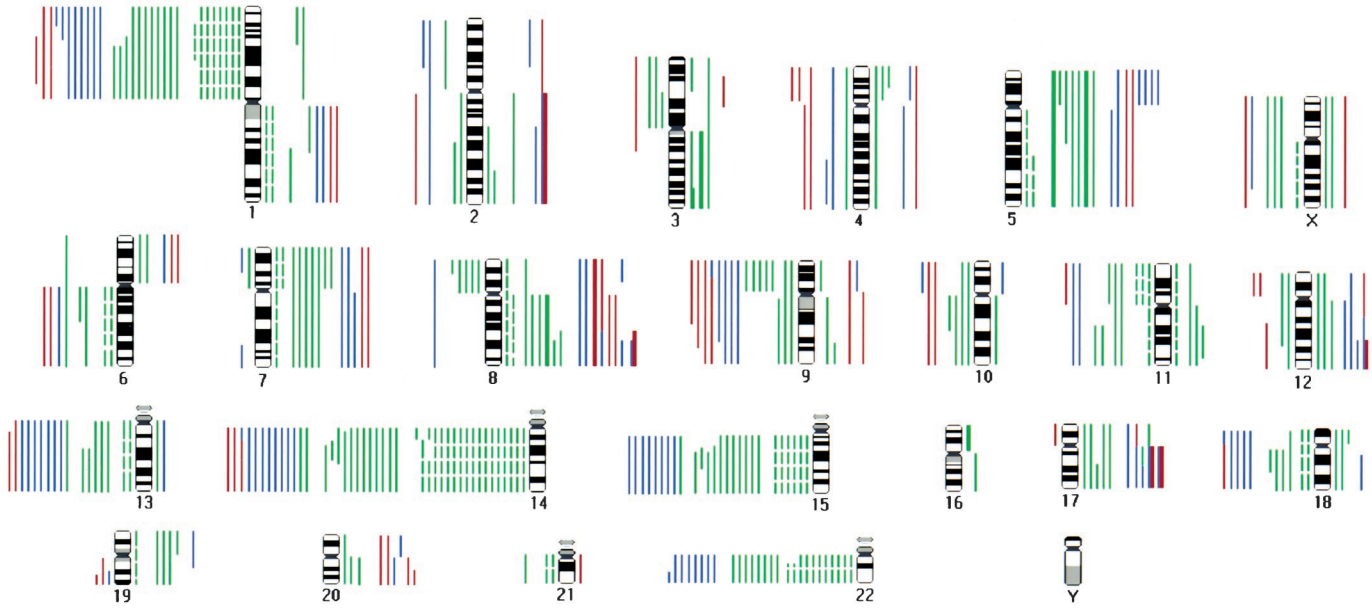


Fig. 1. Summary of DNA copy number gains and losses detected by CGH in 60 GIST patients. Each *bar* represents one patient, regardless of the number of samples available, and the minimal common overlapping regions are presented. Gains are on the *right hand side*, losses on the *left*. *Green broken lines*, benign tumors; *green continuous lines*, malignant primary tumors that had no metastases samples. *Blue lines*, the aberration was seen in the malignant primary tumor and in at least one metastatic sample from the same patient. *Red lines*, changes that were seen on metastatic samples but not in their primary tumors for the same patient.

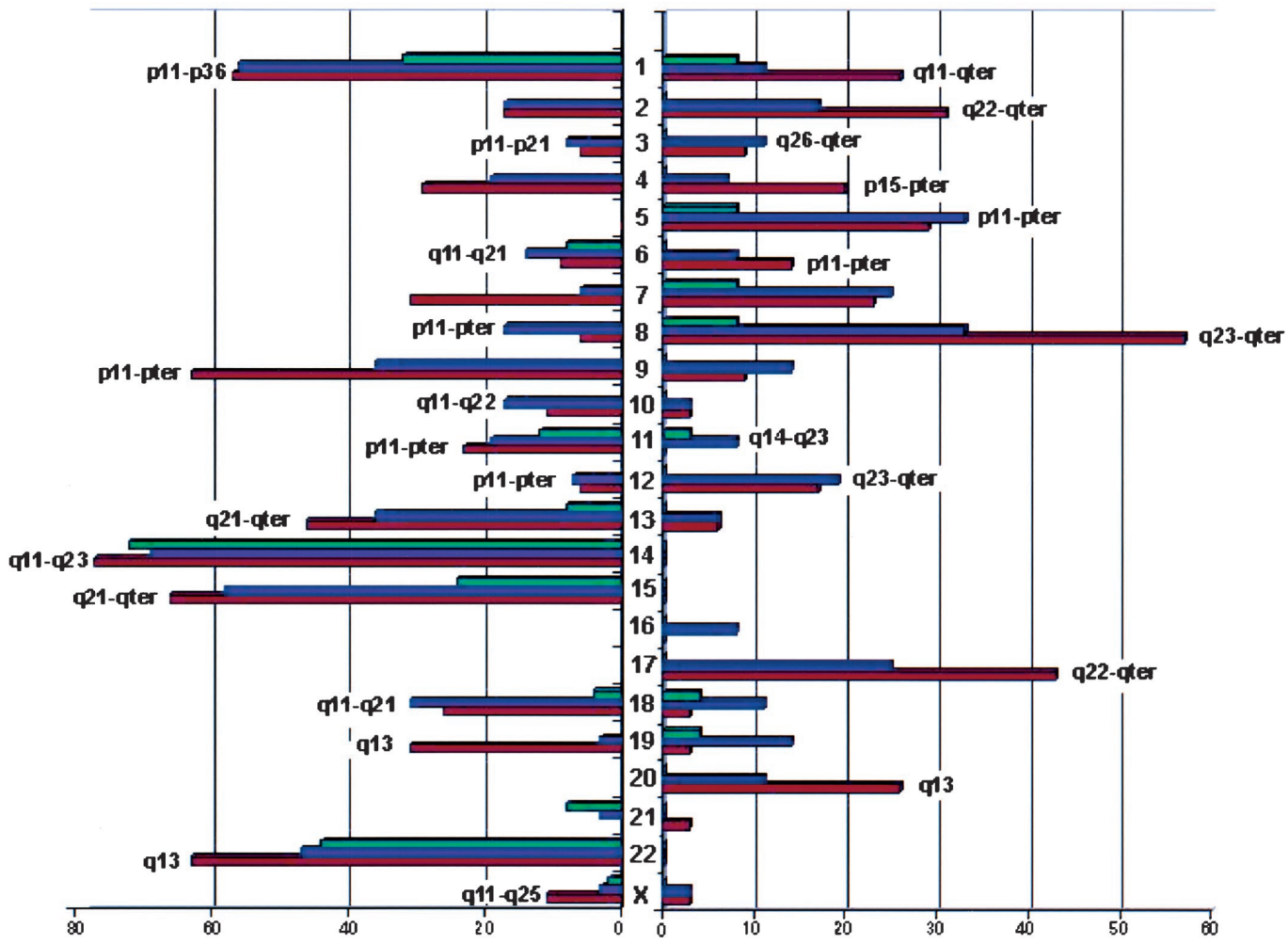


Fig. 2. Comparative frequencies of losses (*left*) and gains (*right*) detected in 24 benign GISTs (*green*), 36 malignant primary GISTs (*blue*), and 35 metastatic GISTs (*red*). Chromosomal numbers are shown in the *middle*, and the minimal common overlapping regions, when applicable, are added to each bar.

gains and high-level amplifications, presents an apparently clear criterion for categorization of these tumors. Accordingly, losses are more likely related to the development of GISTs, whereas accumulation of additional genetic alterations, especially gains/amplicons, is required for malignant transformation and metastatic behavior in GISTs. Absence of gains correlated with benign GISTs with verified good prognosis, whereas presence of gains was more frequent in malignant primary GISTs and their metastases ($P < 0.01$). Therefore, absence of gains can be considered as a good prognostic parameter, which can be used as a new complementary diagnostic criterion for characterization of GISTs.

Gains and high-level amplifications at 5p, 8q, 17q, and 20q and losses in 9p, 13q, 15q, and 19q correlated with malignant primary GISTs and metastatic GISTs. Moreover, the gains at 8q, 17q, and 20q and the losses in 9p were more frequent in metastatic GISTs than in malignant primary GISTs ($P < 0.05$). Therefore, clones with this genetic make-up have a potential capability of developing metastases.

Losses in 1p, 14q, and 22q seen in benign GISTs seem to be unique because they have been reported rarely in other tumors at frequencies as high as observed in GISTs (9). These changes were frequently seen in benign GISTs, indicating that they may be early events in GIST development. The fact that these losses were maintained with tumor progression suggests that they are required for the maintenance of GIST phenotype. The present extended study also supports the notion that these changes may be unique for GIST development. Recently, we reported high-resolution deletion mapping of chromosome 14 in GISTs and identified two possible tumor suppressor loci in 14q11.2 and 14q23 (10).

The gains/high-level amplifications at 5p, 8q, 17q, and 20q as well as the losses in 9p, 13q, 15q, and 19q were detected in many malignant GISTs and their metastases. Such changes have also been reported in several other tumors, such as carcinomas of lung, breast, and ovary, and in squamous cell carcinomas of the head with variable frequencies (reviewed in Refs. 3 and 4). Gains and high-level amplifications at 8q22–q24 and 17q21–qter have been implicated as poor prognostic indicators in breast cancer, ovarian cancer, osteosarcomas, and neuroblastomas (11–14). The gains and high-level amplifications at 8q and 20q correlate with invasiveness in breast cancer (15, 16).

Although these chromosomal regions are known to contain several oncogenes, such as *CMYC* at 8q and *ERBB2* at 17q, the possibility of yet undiscovered oncogenes cannot be ruled out (14). At 20q, several genes have been implicated in breast cancer, and the region is known to harbor specific amplified genes (*AIB1*, *AIB3*, and *AIB4;* Ref. 17). Several candidate genes are located at 20q, *e.g.*, the *PTP1B/PTPN1* gene (20q12), which is involved in growth regulation, and the *MYBL2* gene (20q13), which plays an important role in cell cycle progression. Moreover, the human *cellular apoptosis susceptibility* (*CAS*) gene has been mapped to this same region (18). There are no known oncogenes that map to 5p.

Losses in 9p were associated with recurrences and unfavorable outcome in squamous cell carcinoma of the head and neck (19) and in astrocytic tumors (20). Losses in 13q12–q13 are associated with poor prognosis in familial and sporadic breast cancer (21). Losses in 15q have been observed rarely upon CGH. LOH studies have suggested the presence of a putative tumor suppressor gene in 15q in small cell lung cancer (22). However, there are no data related to its potential prognostic significance or identified tumor suppressor genes.

In summary, our results show that genetic changes can be used as a complementary diagnostic tool for the prognostication of GISTs and for the differentiation between benign and malignant tumors. The increased number of changes and/or increased number of gains correlate with malignant behavior. Furthermore, the genetic changes seen as gains at 5p, 8q, 17q, and 20q and losses in 9p, 13q, 15q, and 19q are new prognostic parameters for GISTs.

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