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PUTATIVE CHROMOSOMAL DELETIONS ON 9P, 9Q AND 22Q OCCUR PREFERENTIALLY IN MALIGNANT GASTROINTESTINAL STROMAL TUMORS

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To characterize the type of genetic alterations in gastrointestinal stromal tumors (GISTs), we performed a comprehensive allelotype study of 14 GISTs (2 benign, 7 borderline and 5 malignant) by polymerase-chain-reaction and loss-of-heterozygosity (PCR-LOH) analysis using 102 microsatellite markers, and compared the results with comparative-genomic-hybridization (CGH) analysis. Among the 38 evaluated chromosomal arms, 16 (42.1%) showed LOH in at least one patient. Most frequent LOH was observed at chromosome 14p and 14q (9/14, 64%) and this was demonstrated in all types of GISTs (50% in benign, 71% in borderline and 80% in malignant). Additional chromosomal deletions were found in several chromosomal arms. Among them, deletions on chromosomal arms of 22q (3/14, 21.4%), 9p (2/14, 14.3%) and 9q (2/14, 14.3%) were the most frequent, and were detected only in malignant GISTs both by PCR-LOH and by CGH analysis. Additionally, 2 malignant GISTs with LOH on 9p showed homozygous deletions in the restricted area of 9p by multiplex PCR-LOH analysis. Thus, several putative chromosomal changes were preferentially present in malignant GISTs but rare in benign and borderline GISTs. These findings suggest that accumulated chromosomal changes may contribute to the progression and/or malignant transformation of GISTs. *Int. J. Cancer* 85:633–638, 2000.
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Most gastrointestinal mesenchymal tumors are composed of uncommitted mesenchymal cells and are designated as gastrointestinal stromal tumors (GISTs) (Lewin *et al.,* 1992; Appelman, 1998). GISTs have been the subject of considerable controversy and debate in the literature regarding their histogenesis, criteria for diagnosis, prognostic features and nomenclature (Lewin *et al.,* 1992; Appelman, 1998; Hjermstad *et al.,* 1987). Immunohistochemical studies have shown GISTs to be characteristically CD34- and c-kit-positive, and negative or variably positive for the other neural and smooth-muscle-cell markers (Kindblom *et al.,* 1998; Miettinen *et al.,* 1995). The expression of c-kit and CD34 is the characteristic feature of the intestinal cell of Cajal (ICC) located in and near the circular muscle layer of the stomach and intestine. On the basis of the studies cited, it has been suggested that these tumors originate from stem cells that differentiate toward ICC because of the morphological and immunohistochemical similarities between ICC and GISTs (Kindblom *et al.,* 1998; Hirota *et al.,* 1998).

The results of molecular genetic changes related to GISTs have been rapidly accumulating. DNA aneuploidy, gain-of-function mutations of the c-*kit* proto-oncogene (Hirota *et al.,* 1998; Nishida *et al.,* 1998; Nakahara *et al.,* 1998; Lasota *et al.,* 1999; Moskaluk *et al.,* 1999) and frequent changes of DNA copy numbers in chromosome 14q and 22q (El-Rifai *et al.,* 1996; Sarlomo-Rikala *et al.,* 1998) have been found in GISTs. Early studies for the changes of DNA copy numbers in GISTs were analyzed by comparative genomic hybridization (CGH) (El-Rifai *et al.,* 1996; Sarlomo-Rikala *et al.,* 1998). CGH analysis is a valuable technique for whole-genome scanning, a procedure that can identify the chromosomal imbalances in entire genomes if the changes exceed 10 Mb. Although CGH study is rapid and less laborious than PCR and loss of heterozygosity (LOH) analysis, allelotype analysis by PCR-LOH study has the advantage of identifying small interstitial deletions, since the microsatellite markers are highly polymorphic and evenly distributed on the chromosomes (Dib *et al.,* 1996). Thus, chromosome analysis of GIST using these 2 methods and comparing the results will give more precise information on the specific changes of DNA copy numbers.

Among the known DNA-copy-number changes, the loss of chromosome 14q is very common in GISTs. This change is not found in leiomyoma or schwannoma, suggesting that GIST is a different type of tumor from leiomyomas or schwannomas, both phenotypically and genetically (Sarlomo-Rikala *et al.,* 1998). Loss of chromosome 14q is known to be frequent in benign, borderline and malignant tumors, and is thus regarded as an early change in GISTs (El-Rifai *et al.,* 1996). Other genetic changes of DNA copy numbers are relatively infrequent, and are not well characterized. The identification of common DNA-copy-number changes and small interstitial deletions in specific chromosomal arms in GISTs should provide understanding of genetic changes related to the development and progression of GIST. We therefore carried out an allelotype study for 14 cases of GISTs and compared the results with CGH analysis.

MATERIAL AND METHODS

Tissue samples

Fourteen GISTs occurring in the stomach were included in this study. All the cases were identified prospectively and consecutively in the Department of Pathology at Yonsei University Medical Center between September 1995 and November 1998 for a study of molecular markers in GISTs. Information was obtained from chart reviews and from clinicians to determine demographic and tumor sites. The patients included 5 females and 9 males, ranging in age from 35 to 78 years (Table I).

For DNA extraction, tumors and adjacent non-tumorous mucosal tissues were obtained immediately after surgical excision. The selected tissues were rapidly frozen in liquid nitrogen and stored at −70°C until the DNA was isolated. To enrich the tumor-cell population, more than 90% of tumor-cell areas were selected from the hematoxylin-eosin stained slides by the cryostat microdissection technique. Genomic DNA was prepared by the sodium-dodecyl-sulfate-proteinase-K and phenol-chloroform extraction method.

Pathological analysis

Conventional pathologic parameters (tumor size, tumor number, differentiation) were examined prospectively without knowledge of the molecular data. The GISTs were divided into 3 groups according to the criteria of Lewin *et al.* (1992). The guidelines for the diagnosis of malignancy of gastric stromal tumors are composed of 2 unequivocal factors (histologically confirmed metasta-

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TABLE I – CLINICOPATHOLOGICAL FEATURES OF GASTROINTESTINAL STROMAL TUMORS

Case number	Tumor size(cm)	Tumor type	Cell type	Anatomic site	Mitotic index[1]	Immunohistochemical result			
						S100	α-smooth muscle	CD34	c-kit
1	2.5	benign	spindle	body	0	−	−	+	+
2	4	benign	spindle	body	1	−	−	+	+
3	7	borderline	spindle	body	0	−	−	+	+
4	5	borderline	epitheloid	antrum	4	−	+	+	+
5	4	borderline	spindle	fundus	11	−	+	+	+
6	8	borderline	spindle	fundus	0	−	−	+	+
7	9	borderline	spindle	body	2	−	−	+	+
8	5.5	borderline	epithelioid	fundus	2	−	−	+	−
9	5	borderline	spindle	body	2	−	−	+	+
10	12	malignant	spindle	body	7	−	−	+	+
11	10	malignant	epithelioid	body	4	−	+	−	+
12	17	malignant	epithelioid	body	172	−	−	+	+
13	22	malignant	spindle	antrum	9	−	−	+	+
14	3.7	malignant	spindle	fundus	98	−	−	+	+

[1]Number of mitoses per 50 high-power field. +, expression; −, no expression.

sis, and invasion of adjacent organs) and 5 high-risk factors (larger than 5.5 cm in diameter, more than 5 mitoses per 50 high-power field, presence of tumor necrosis, nuclear pleomorphism, dense cellularity, microscopic invasion of lamina propria or blood vessels and the presence of alveolar or cell balls in the epithelioid variant). Tumors having more than 1 unequivocal or 2 high-risk features were categorized as malignant GISTs, tumors having only 1 high-risk feature were categorized as borderline (stromal tumor of uncertain malignant potential, STUMP) and tumors having no unequivocal or high-risk features were categorized as benign GISTs. By these criteria, 2 cases were categorized as benign, 7 cases as STUMP and 5 cases as malignant GIST. Histologically, 10 cases were categorized as spindle-cell type and the remaining 4 cases as epithelioid-cell type.

Immunohistochemical analysis

Formalin-fixed and paraffin-embedded tissues were sectioned in 6-μm thickness. De-paraffinization and re-hydration were performed using xylene and alcohol. The sections were treated with 0.3% hydrogen peroxidase for 3 min and blocking antibody for 30 min. The primary antibodies used included those to CD34 (clone QBEMD-10, 1:80 dilution; Monosan, The Netherlands), α-smooth-muscle actin (clone HHF35, 1:50 dilution; DAKO, Glostrup, Denmark), S100 (rabbit polyclonal, 1:1,500 dilution; DAKO) and c-kit (rabbit polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA). Avidin-biotin-complex methodology was used. The chromogen was diaminobenzidine and counterstaining was done with hematoxylin. For evaluation of these gene products, definite cytoplasmic and plasma membrane staining was regarded as positive.

PCR-LOH analysis

A total of 102 microsatellite markers covering 38 chromosome arms were selected and obtained from Research Genetics (Huntsville, AL). They are shown in Table II. Multiplex PCR reactions were carried out in a mixture of 20 μl containing 1.5 mM $MgCl_2$, 20 pmol primer, 0.2 mM each dATP, dGTP, dTTP, 5 μM dCTP, 1 μCi [α-^{32}P] dCTP (3000 Ci/mmol; NEN DuPont, Boston, MA), 50 ng of sample DNA, 1× PCR buffer and 1.25 U Taq polymerase (GIBCO-BRL, Grand Island, NY). After de-naturation at 95°C for 5 min, DNA amplification was performed for 25 to 30 cycles consisting of de-naturation at 95°C for 30 sec, primer annealing at 55 to 60°C for 30 sec and elongation at 72°C for 15 sec.

Amplified DNA was diluted 2-fold with stop solution (95% formamide, 20 mM EDTA, 0.05% xylene cyanol and 0.05% bromophenol blue). Then 3 μl of amplified product were loaded onto 6% polyacrylamide gel containing 5.6 M urea. The gel was dried on filter paper and exposed to Kodak XAR-5 film (Kodak, Rochester, NY) at −70°C. LOH was scored when the band intensity of one allelic marker decreased significantly (more than 70% loss) in tumor DNA compared with that in normal DNA (Fig. 1). LOH on each chromosome arm was further divided into high (more than 50% of informative cases), moderate (30–50%) and low (less than 30%) according to the frequency of allelic loss. Fractional allelic loss (FAL) was defined as the number of chromosomal arms showing allelic loss divided by the total number of informative chromosomal arms in a tumor, as described by Vogelstein *et al.* (1989).

Comparative genomic hybridization and digital image analysis

Comparative genomic hybridization (CGH) was performed using direct fluorochrome-conjugated dUTPs. Genomic DNA samples from tumors were labeled with Spectrum Green dUTP (Vysis, Downers Grove, IL), and reference genomic DNA was labeled with Spectrum Red dUTP (Vysis) by the nick-translation technique, to obtain DNA fragments ranging from 300 to 3,000 bp. Labeled tumor and reference DNA (200–400 ng) and 10 μg of unlabeled human Cot-1 DNA (Vysis) were ethanol-precipitated and re-suspended in 10 μl of hybridization buffer (50% formamide, 10% dextran sulfate, 2× SSC). The hybridization mixture was de-natured at 73°C for 5 min, then immediately hybridized onto normal metaphases de-natured in 70% formamide/2× SSC at 73°C for 2 min. Hybridization was carried out at 37°C for 72 hr. After hybridization, the slides were washed 3 times in 50% formamide/2× SSC (pH 7.0), 3 times in 0.2× SSC at 45°C, followed by washes in 2× SSC, 0.2× SSC and distilled water at room temperature for 10 min each. After drying, the slides were counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) in anti-fade solution. CGH hybridization was analyzed with an Olympus fluorescent microscope and the Cytovision image-analysis system (Applied Imaging, Sunderland, UK). The individual DAPI, Spectrum Green, Spectrum Red images were subsequently captured in gray digital images from 10 to 20 methaphases in each hybridization, then digitally pseudocolored in blue, green and red. Normal male DNA and DNA from tumor cell lines with known aberrations were used as control test DNA. Green-to-red intensity-ratio profiles were calculated for each chromosome, and threshold values defining gains and losses were set at 1.25 and 0.75 respectively.

RESULTS

Immunohistochemical features of gastrointestinal stromal tumors

Examination of 14 gastrointestinal stromal tumors for expression of CD34 and c-kit, known reliable markers for GISTs, revealed that 12 cases (86%) were positive both for CD34 and for c-kit and the remaining 2 cases were positive for only one of these 2 markers. α-smooth-muscle actin was focally present in 3 cases (21%), and no cases were positive for S100 protein.

TABLE II – SELECTED MICROSATELLITE MARKERS AND FREQUENCY OF LOH ON EACH CHROMOSOME ARM

Chromosome arm	Locus	Allelic loss/informative case (%)	Chromosome arm	Locus	Allelic loss/informative case (%)
1p	D1S496		10p	D10S591	
	D1S209	1/13 (7.7)		D10S191	1/14 (7.1)
1q	D1S431		10q	D10S185	
	D1S412			D10S209	
	D1S237			D10S1072	
	D1S446	0/14		D10S555	1/14 (7.1)
2p	D2S165		11p	D11S988	
	D2S123			D11S1308	
	D2S286	0/14		D11S935	1/14 (7.1)
2q	D2S114		11q	D11S918	
	D2S156			D11S898	
	D2S155	1/14 (7.1)		D11S1320	0/11
3p	D3S1260		12p	D12S93	
	D3S1566	1/13 (7.7)		D12S358	0/14
3q	D3S1310		12q	D12S85	
	D3S1288	1/14 (7.1)		D12S327	
4p	D4S1608	0/8		D12S343	0/14
4q	D4S411		13q	D13S144	
	D4S1615			D13S121	2/12 (16.7)
	D4S1566		14p	D14S582	
	D4S1554	0/14		D14S152	9/14 (64.2)
5p	D5S486		14q	D14S281	
	D5S395	0/14		D14S268	
5q	D5S424			D14S267	
	D5S489			D14S51	9/14 (64.2)
	D5S494		15q	D15S153	
	D5S625	0/14		D15S211	0/14
6p	D6S344		16p	D16S521	
	D6S289			D16S292	1/14 (7.1)
	D6S452	0/14	16q	D16S752	
6q	D6S278			D16S516	
	D6S292			D16S505	0/13
	D6S297	1/13 (7.7)	17p	D17S520	
7p	D7S481	0/7		D17S969	
7q	D7S645			D17S953	1/14 (7.1)
	D7S487		17q	D17S250	
	D7S684	0/14		D17S939	0/12
8p	D8S264		18p	D18S40	0/12
	D8S552	1/14 (7.1)	18q	D18S46	
8q	D8S279			D18S51	0/13
	D8S257		19q	D19S178	
	D8S555	1/14 (7.1)		D19S246	
9p	D9S162			D19S418	0/13
	D9S1846		20q	D20S27	
	D9S171			D20S108	
	D9S165	2/14 (14.3)		D20S109	
9q	D9S152			D20S171	0/13
	D9S280		21q	D21S11	
	D9S176			D21S1255	
	D9S195	2/14 (14.3)		D21S231	0/14
			22q	IL2RB	
				D22S685	3/14 (21.4)

Frequency of LOH on each chromosomal arms

We screened 14 gastrointestinal stromal tumors for LOH at every chromosomal arm with 102 microsatellite markers. The average informativeness per marker was 68.5% (range, 9.2 to 100%). Among the 38 chromosomal arms, 18 (1p, 2q, 3p, 3q, 6q, 8p, 8q, 9p, 9q, 10p, 10q, 11p, 13q, 14p, 14q, 16p, 17p, 22q) showed LOH for at least 1 patient, while the remaining 20 chromosomal arms (1q, 2p, 4p, 4q, 5p, 5q, 6p, 7p, 7q, 11q, 12p, 12q, 15q, 16q, 17q, 18p, 18q, 19q, 20q, 21q) showed no LOH. Among all 14 patients, 9 (64%) showed LOH on at least 1 chromosomal arm (Table II). Representative examples of autoradiograms scored as LOH are shown in Figure 1. The frequency of LOH on each chromosome arm is shown in Figure 2*a*. Frequency LOH (>50%) was found on chromosome 14 (64%) and other chromosome arms had a low LOH frequency of less than 30%.

Fractional allelic loss

Fractional allelic loss (FAL), defined as the number of chromosome arms showing allelic loss divided by the total number of informative chromosomal arms in a tumor (Vogelstein *et al.,* 1989) was calculated for all 14 tumors. The FAL value varied among 14 cases, ranging from 0 (0 of 37 informative arms) to 0.24 (9 of 37 informative arms) with a median value of 0.069 and a mean of 0.076, indicating that 7.6% of chromosomal arms had LOH in our cases. The malignant GISTs showed higher FAL values than benign and borderline GISTs (0.14 *vs.* 0.004, $p < 0.04$ by Wilcoxon's rank-sum test).

Homozygous deletions on chromosome 9p

We found 2 cases of homozygous deletions in D9S1846 markers on chromosome 9p by multiplex PCR-LOH analysis (Fig. 3). Both cases (case 13, 14) were malignant GIST. These 2 cases showed LOH on the entire area of 9p in one chromosomal arm and also

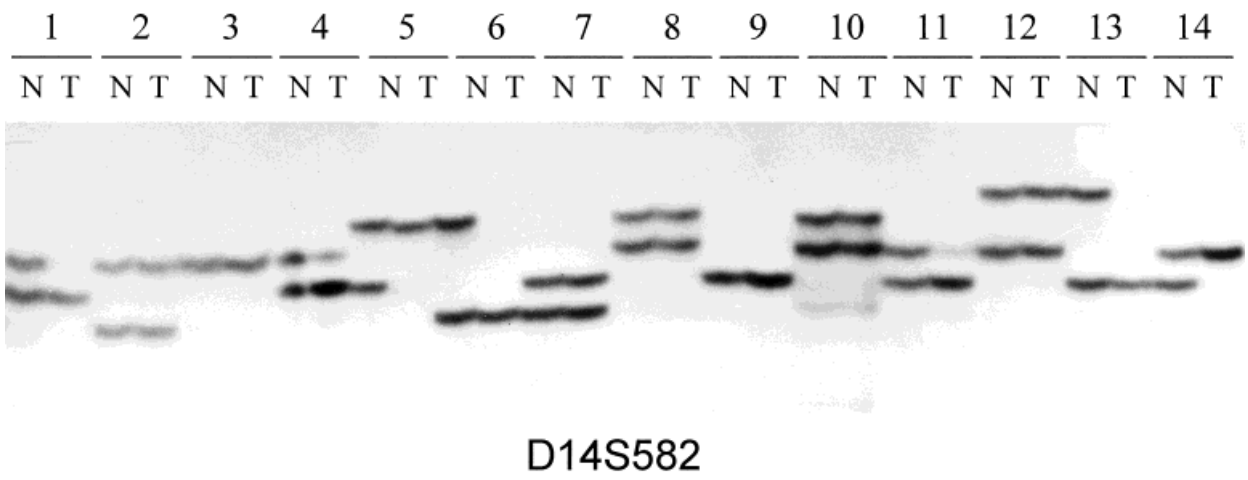

FIGURE 1 – Illustration of loss-of-heterozygosity analysis with microsatellite marker D14S582. The stromal tumor (T) and corresponding non-tumoral tissue (N) are shown with microsatellite marker indicated at the bottom. Cases 1 and 2 are benign, cases 3 to 9 are borderline and cases 10 to 14 are malignant GIST. Tumor cases 1, 4, 6, 11 and 13 exhibit loss of the upper allele. Tumor cases 5 and 14 exhibit loss of the lower allele.

showed a restricted area of homozygous deletions in the other chromosomal arm.

Chromosomal copy number aberrations by CGH analysis

CGH analysis was also performed in 14 gastrointestinal stromal tumors. Among these, 10 stromal tumors exhibited several genetic imbalances (range, 0–7/case; mean number 2.65/case); chromosomal losses were more frequent than gains. A schematic summary of all copy number aberrations is shown in Figure 2*b*. The chromosomal arms most often under-represented were 14q (64%, 9/14) and 22q (21.4%, 3/14). Under-representation of 14q and 22q involved the entire long arm in all cases affected (Fig. 2*b*). The entire deletion on chromosome 14q was confirmed by PCR-LOH study using 4 microsatellite markers.

Other commonly under-represented segments in this series were 9q (14.3%, 2/14), 9q (14.3%, 2/14) and 13q (16.7%, 2/12), and under-representation of these chromosomal arms was also involved in all chromosomal arms. In addition, under-representation involving the entire segment of 1p and interstitial losses of 8p, 10p and 10q were observed in only 1 case for each deletion (Fig. 2*b*). Over-representation of a chromosome arm was also observed infrequently: 3 cases in 18p and 19p, 2 cases in chromosome 4p, 4q and 22q, and 1 case in 2p, 2q, 5q11–21, 8q12–23, 12q, 16q, and 20p.

Comparison of allelotype analysis by PCR-LOH and of chromosomal copy numbers by CGH

In our GISTs, both methods gave the same results for chromosomal losses. Most of the chromosomal arms with LOH showed wide scope deletions usually covering the entire chromosomal arms, and they were detected by CGH (Table II, Fig. 2). However, deletions on chromosome 14p in 9 cases and 2 homozygous deletions on 9p detected by multiplex PCR-LOH analysis were not detected by CGH. Most of the chromosomal gains detected by CGH presented as allelic imbalance by PCR-LOH analysis (Fig. 4); however, these were not interpreted as LOH, since LOH was scored when one allele was almost totally missing (more than 70% loss).

DISCUSSION

In this study, 14 cases of fresh frozen gastrointestinal stromal tumors were investigated for the characterization of chromosomal abnormalities by PCR-LOH and CGH methods. We confirmed earlier findings that frequent losses on chromosome 14q were present in most benign, borderline and malignant GISTs. In addition, we found that all the GISTs with 14q deletion also had 14p deletion. We found additional novel losses and gains on several chromosomal arms and homozygous deletions on chromosome 9p in malignant GISTs.

The frequent loss of 14q was shown by CGH analysis (El-Rifai *et al.,* 1996), and subsequent experiment demonstrated that this change was rare in other types of mesenchymal tumors (Sarlomo-Rikala *et al.,* 1998). In agreement with these reports, we demonstrated frequent losses (9/14, 64%) on the entire long arm of chromosome 14 in benign, borderline and malignant GISTs. However, we demonstrated that all cases with 14q deletion also had 14p deletion by PCR-LOH analysis. The deletion of 14p was not described in earlier chromosomal studies with CGH analysis (El-Rifai *et al.,* 1996; Sarlomo-Rikala *et al.,* 1998), possibly because CGH analysis cannot detect chromosomal changes in the satellite chromosomal arms (Kallioniemi *et al.,* 1994). The monosomy of chromosome 14 was detected in one GIST by karyotype analysis (Marci *et al.,* 1998). The mechanism for this specific deletion of chromosome 14 in GISTs and its role in tumor development are unknown. Whatever the mechanism, this finding, in association with currently reported results that most GISTs express c-kit protein, may be useful in diagnosis.

In addition to chromosome-14 loss, we found other losses and gains on 18 chromosome arms, but the frequency was low (<30%). Among these changes in chromosomal arms, loss in DNA copy numbers at 1p, 15q and 22q, and allelic gains in chromosome 5q, 19p and 8q were reported by El-Rifai *et al.* (1996). The other chromosomal changes in 12 chromosomal arms (4q, 6q, 8p, 9p, 9q, 10q, 12q, 13q, 16q, 18p, 18q and 20p) were detected by us. Interestingly, these alterations were absent in benign and rare in borderline GISTs. In malignant GISTs, the frequency of alterations in 14p, 14q and 22q was high (>50%) and in 9p and 9q was moderate (30 to 50%), indicating that alterations of chromosomal arms other than chromosome 14, especially 9p, 9q and 22q, are accumulated in malignant GISTs. These findings suggest that additional chromosomal changes occur as late events related to tumor progression and malignant transformation.

In agreement with other studies, most of the abnormal areas on the chromosome were extensive, and entire chromosomal arms were usually deleted. Among the newly found abnormal chromosomal areas, losses of chromosomal arms on 9p and 10q are of particular interest. The case with 10q deletion (case 4) showed a

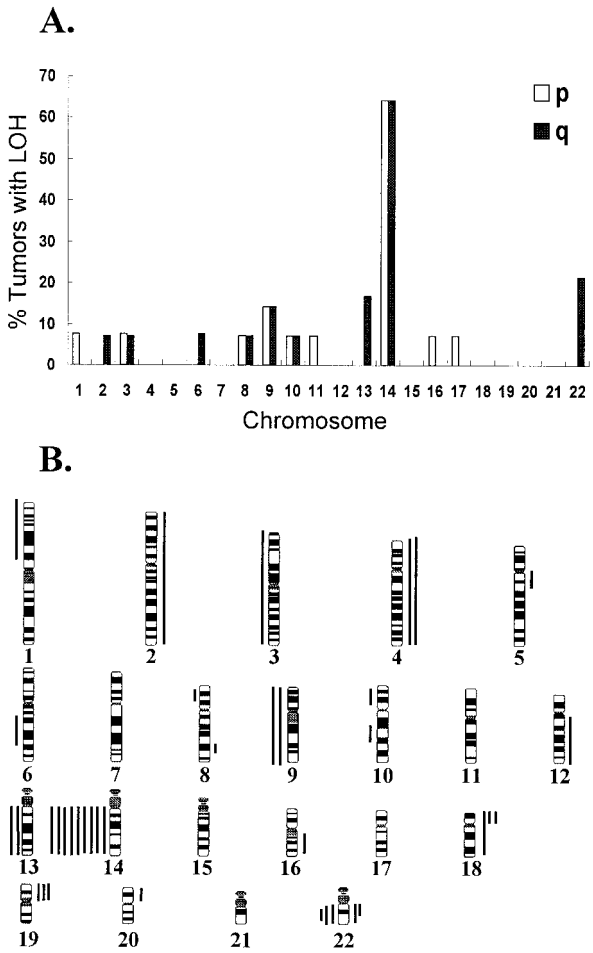

FIGURE 2 – Results of LOH and CGH imbalance analysis. (*a*) Frequency of LOH on each chromosomal arm in 14 gastrointestinal stromal tumors. The microsatellite markers used are listed in Table II. p denotes short arm and q denotes long arm. (*b*) Summary of CGH imbalances detected in 14 gastrointestinal stromal tumors. Vertical lines on the left of each chromosome idiogram represent loss of chromosomes, whereas vertical lines on the right correspond to chromosomal gains.

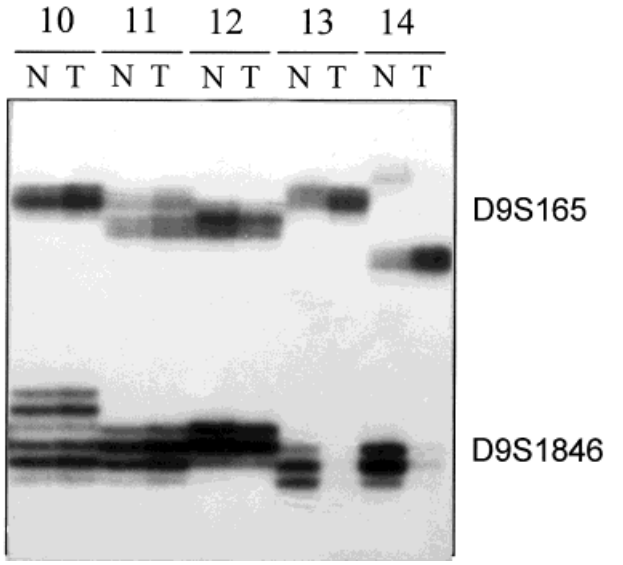

FIGURE 3 – Representative autoradiograph of multiplex PCR-LOH analysis with D9S165 and D9S1846 microsatellite markers. LOH was demonstrated in cases 13 and 14 by D9S165 markers. The homozygous deletions were demonstrated by a complete loss of amplified products with D9S1846 markers in cases 13 and 14.

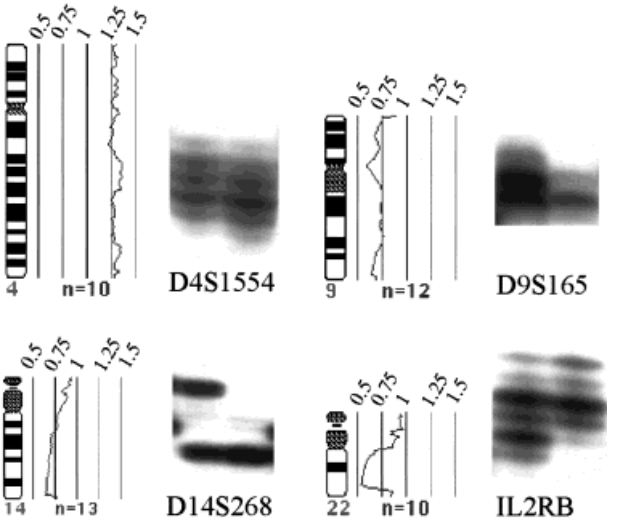

FIGURE 4 – Comparison of the results of CGH and PCR-LOH analyses from case 13. Green-to-red intensity ratio was calculated for each chromosome, and threshold values defining gains and losses were set at 1.25 and 0.75. n denotes number of metaphase chromosome images captured after CGH. The microsatellite markers used are shown at the bottom. Note that over-representation of chromosome 4 detected by CGH analysis shows as allelic imbalance by PCR-LOH analysis, whereas under-representation of chromosomes 9, 14 and 22 detected by CGH analysis shows as the loss of one allele by PCR-LOH analysis.

relatively small area of deletions (10q21–26) and this area harbors the *pten* gene (10q23), a tumor-suppressor gene identified by Li *et al.* (1997). However, this phenomenon might be incidental, since the incidence of loss of 10q is quite low (1/14, 7.1%) in GISTs. Two malignant GISTs (case 13 and 14) with 9p deletion showed the entire deletion of 9p in one chromosomal arm and had homozygous deletions in the restricted area of 9p from D9S1846 to D9S171 encompassing 5.5 cM, corresponding approximately to a 3.71 Mb when 1 cM is expected to cover 675 kb of physical distance (Krauter *et al.,* 1995). Interestingly, these small areas of homozygous deletions contain the $p16^{INK4A}$ gene, a well-known tumor-suppressor gene involved in multiple human tumors (Liggett and Sidransky, 1998). The present PCR-LOH study for allelotype analysis also shows homozygous deletions in the restricted area of certain chromosomal arms in GISTs. The role of the tumor-suppressor gene in malignant GIST has not yet been reported. Since homozygous deletions are among the mechanisms for complete inactivation of the tumor-suppressor gene, it can be concluded that inactivation of $p16^{INK4A}$ may play a role in the progression of some malignant GISTs.

Our allelotype study, by using multiplex PCR-LOH and CGH analysis, permitted us to identify detailed chromosomal changes in GISTs. The chromosomal deletions covering large areas were identified identically in PCR-LOH and CGH analysis. However, homozygous deletions covering a small area on 9p and the entire deletion of chromosome 14p, one of the acrocentric chromosomal arms, were not found in CGH analysis. Although most of the chromosomal gains are demonstrated as allelic imbalance in PCR-LOH analysis, the results of CGH were more objective in identifying specific chromosomal gains. From these findings it can be concluded that the use of both methods for allelotype analysis

provided more accurate evaluation of chromosomal status in tumors.

In summary, a comprehensive allelotype study of GIST was completed using PCR-LOH and CGH analysis. We have demonstrated that the deletion of chromosome 14 is the most frequent chromosomal alteration in GISTs. We also found several putative chromosomal-arm alterations in malignant GISTs, while such changes were rare in benign and borderline GISTs. Homozygous deletions on the short arm of chromosome 9 were observed in only 2 malignant GISTs. These findings suggest that genetic changes are accumulated during the progression of GISTs.

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