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Loss of heterozygosity on chromosome 22q in gastrointestinal stromal tumors (GISTs): a study on 50 cases

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Mutational activation of KIT or PDGFRA is considered an early step in pathogenesis of gastrointestinal stromal tumors (GISTs); however, other nonrandom genetic changes have also been identified. At least three common regions of deletions on chromosome 22q, which may harbor putative tumor suppressor genes, have been defined. However, mapping of these regions has been inconsistent. It has also been speculated that GI autonomous nerve tumors (GANTs), GISTs with ultrastructural features suggestive of autonomic nerve differentiation, are characterized by a specific deletion involving 22q13 cytogenetic region. This study was undertaken to evaluate loss of heterozygosity (LOH) on chromosome 22q in 50 GISTs, including 10 GANTs. Four tumors were incidental minimal lesions $\leq$10 mm in diameter. LOH was evaluated using 20 PCR-based microsatellite markers and capillary gel electrophoresis. In all, 15 (30%) cases showed LOH of more than 75% of informative markers, suggesting loss of chromosome 22q. A total of 24 GISTs (50%) revealed LOH of one to seven informative markers clustered in different loci suggesting simultaneous involvement of different regions. The highest frequency of LOH was seen at D22S922 and D22S425, mapped to 22q13.33 and 22q11.22, respectively. However, LOH at other regions including IL2RB and NF2 locus was also found. No *NF2* mutations were identified in four analyzed tumors. LOH on chromosome 22q was more frequent among intestinal than among gastric GISTs; however, there was no difference between LOH pattern seen in tumors defined by different histologic, ultrastructural (GANT) and molecular features (KIT and PDGFRA mutations). Although minimal GISTs revealed LOH on chromosome 22q, there was a higher LOH frequency in malignant than in benign tumors. An isolated LOH at D22S425 was equally found in both benign and malignant tumors. These observations may suggest that LOHs on chromosome 22q in GISTs play a role in early stages of tumor formation as well as in late tumor progression.
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Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal (GI) tract, previously often diagnosed as benign or malignant smooth muscle tumors, GI autonomic nerve tumors (GANTs) and schwannomas.[1] A great majority of GISTs express KIT and have gain-of-function KIT or PDGFRA mutations. Mutational alteration of KIT or PDGFRA leads to ligand-independent activation (phosphorylation) of these tyrosine kinase receptors, has a transforming effect *in vitro* and is considered to be an early molecular event leading to the development of GISTs.[2,3] Germline KIT or PDGFRA mutations similar to those identified in sporadic GISTs have been reported in human familial GIST syndromes.[4,5]

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Moreover, features of human familial GIST syndrome were reproduced in a mouse model by introduction of KIT activating mutation.[6]

Although mutational activation of KIT or PDGFRA plays an important role in GIST pathogenesis, other changes, mostly losses of genetic material, have been documented in primary tumors.[7–16] Total or partial loss of chromosome 22 has been found in benign and malignant GISTs indicating that this change might play a role in GIST tumorigenesis.[9] However, some of the studies linked loss of heterozygozity (LOH) on chromosome 22q to tumor progression and malignant outcome.[12,15] A comparative genomic hybridization (CGH) study defined 22q12–qter as a common region of deletion in GISTs,[9] while fluorescence *in situ* hybridization (FISH) study based on five GISTs with ultrastructural features suggestive of autonomic nerve differentiation (GISTs/GANTs) identified the common region of deletion at 22q13.[16] Based on one LOH study, *NF2* was indicated as a possible target of deletions on chromosome 22q in GISTs; however, no convincing evidence of 'Knudson type' two hit inactivation of *NF2* has been found.[11] Moreover, two frequent LOH regions on chromosome 22, separated from NF2 locus, were recently identified in malignant GISTs.[15]

This study was undertaken to address some of the above-mentioned controversies emerging from the previously published investigations.[11,15,16] A group of 50 well-characterized GISTs including small incidentally detected tumors and GISTs/GANTs was evaluated for LOH on chromosome 22q to identify common region/regions of deletions, which may harbor genes important for GISTs pathogenesis.

Materials and methods

Tissue Material

Samples of the tumor and corresponding normal tissue, demographic and clinical data were obtained from the files of the Armed Forces Institute of Pathology, Washington, DC, USA; the Department of Pathology, New York University Medical Center, New York, NY, USA, the Haartman Institute of the University of Helsinki, Finland, from the Medical College of Georgia and Veterans Affairs Medical Center, Augusta, Georgia; the Collegium Medicum of the Jagiellonian University, Krakow Poland and the Otto-von-Guericke University, Magdeburg, Germany.

Tumors were diagnosed as GISTs using previously established histological, immunohistochemical and molecular genetic criteria.[1] Also based on published criteria,[17,18] tumor size and mitotic activity were used to evaluate the likelihood of malignant behavior (Table 1). All GISTs were classified into six prognostic groups. Group 1 and 2 tumors were considered benign or of very low malignant potential. GISTs classified into groups 3–6 represented tumors of uncertain malignant potential to the high-grade sarcomas (group 6).

Table 1 Tumor size and mitotic criteria used to evaluate the clinical behavior of GISTs

Group	*Size (cm)*	*Mitosis (per 50 HPF)*	*Predicted clinical behavior*
1	≤ 2	≤ 5	Benign
2	>2 but ≤ 5	≤ 5	Benign or very low malignant potential
3	>5	≤ 5	Uncertain malignant potential
4	≤ 2	>5	Probably malignant
5	>2 but ≤ 5	>5	Malignant
6	>5	>5	Malignant

Genetic Studies

Tumor and normal tissue samples were microdissected from formalin-fixed paraffin embedded (FFPE) tissue blocks and evaluated for possible cross-contamination to ensure purity of DNA samples. DNA was extracted as previously described.[19] LOH was evaluated by PCR amplification of 20 microsatellite markers mapped to the chromosome 22q. Primer sequences were obtained from human genome microsatellite marker databases linked to the webpage of the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov).

PCR amplification was performed using standard conditions recommended by Applied Biosystems (www.appliedbiosystems.com). PCR products were analyzed on ABI PRISM® 310 Genetic Analyzer following the Applied Biosystems procedure. The LOH was defined as recommended by PE Biosystems and previously reported.[20] A ratio of the peak high values (fluorescence intensity) between longer and shorter alleles was calculated for the normal and tumor tissues. To obtain LOH value, an allele ratio from normal tissue was divided by an allele ratio from tumor tissue. The values ≤ 0.5 and ≥ 1.5 were considered to represent loss of heterozygosity.

Marker positions were established based on Human Chromosome 22 Sequence Map, deCODE Map and Marshfield Map (www.ncbi.nlm.nih.gov). Table 2 shows the orders of chromosome 22q microsatellite and FISH markers used in this and other studies.[11,15,16]

In eight cases that were noninformative for D22S929 microsatellite marker mapped to the first *NF2* intron, additional single nucleotide polymorphic (SNP) *NF2* markers were evaluated by PCR amplification and direct sequencing as previously described.[21] Moreover, *NF2* coding sequences (exons 1–16) were screened for mutations by PCR amplification and direct sequencing in 4 cases, following previously published procedures.[22]

Table 2 Orders of chromosome 22q microsatellite markers used in current and previous studies[11,15] on LOH in GISTs

Order of markers				*Position*			
NCBI	*Current study*	*Fukosawa* et al	*Pylkkanen* et al	*Cytogenetic*	*Sequence Map (bp)*	*deCODE Map (cM)*	*Marshfield Map (cM)*
D22S420	D22S420		D22S420	22q11.21	16234030–16234181	2.96	4.06
D22S427	D22S427			22q11.21	16965930–16966031	5.8	8.32
D22S311			D22S311				
D22S264	D22S264	D22S264		22q11.21	19097786–19097989		
D22S446		D22S446		22q11.21	20343712–20343913		14.44
D22S425	D22S425			22q11.22	21407123–21407320	15.46	13.57
D22S303			D22S303	22q11.22	21599366–21599581		
D22S257	D22S257			22q11.23	21892983–21893115	16.8	17.71
			D22S446[a]				
D22S345	D22S345			22q11.23	22813141–22813272		
			D22S689[a]				
CRYB2A			CRYB2A	22q11.23	23941939–23952383		
			D22S425[a]				
D22S421	D22S421			22q11.23	24276744–24276908		21.47
D22S315		D22S315		22q12.1	24340416–24340608		
D22S310	D22S310			22q12.1	24952777–24952966	24.38	23.37
D22S689				22q12.1	27181014–27181237	32.92	28.57
D22S929	D22S929	D22S929	D22S929	22q12.2	28348625–28348762		
D22S268	D22S268			22q12.2	28882755–28883007		
			D22S685[a]				
D22S280	D22S280	D22S280	D22S280	22q12.3	31533927–31534146	37.03	31.30
D22S685				22q12.3		38.79	32.39
		D22S268[a]					
D22S304	D22S304			22q12.3	33695237–33695347		
D22S277	D22S277	D22S277		22q12.3	34543413–34543578		36.22
			D22S445[a]				
D22S683			D22S683	22q12.3	34785740–34785917		36.22
D22S283	D22S283			22q12.3	35022762–35022895		38.62
IL2RB	IL2RB			22q12.3	35789123–35789257		
D22S445				22q13.1		45.22	45.82
D22S284	D22S284			22q13.1	38559962–38560059	49.01	46.42
D22S423		D22S423		22q13.1	38625239–38625469	49.14	46.42
D22S270	D22S270			22q13.2	41284472–41284608	49.92	
D22S418	D22S418			22q13.2	41649864–41650000		48.19
D22S274		D22S274		22q13.31	43545718–43545923	56.47	51.54
D22S928	D22S928			22q13.31	43752015–43752176	57.28	52.08
D22S922	D22S922			22q13.33	47393317–47393437	67.89	60.61

[a]Markers previously mapped at the different locations. Cytogenetic, Sequence Map, deCODE Map and Marshfield Map positions are according to the NCBI human genome map (http://www.ncbi.nlm.niv.gov) and GeneLoc formarly known as The Unified Database for Human Genome Mapping (http://genecards.weizmann.ac.il/geneloc).
bp: base pair; cM: centimorgan.

Genomic sequences of *KIT* exons 9, 11, 13 and 17 and *PDGFRA* exons 12 and 18 were evaluated for mutations by PCR amplification and direct sequencing as previously described.[23–25]

In one case, tumor tissue was enzymatically disaggregated and cultured for cytogenetic analysis as previously reported.[26] Chromosomes and chromosomal abnormalities were identified using GTW banding and described according to the International System of Human Cytogenetic Nomenclature.[27]

Results

Demographic, Clinical and Pathologic Features

All demographic, clinical and pathologic data are summarized in Table 3. The patient age varied from 29 to 86 years with median age of 59 years. The male to female ratio was 32:18. There were 31 gastric, 10 small intestinal, two colonic, four rectal and two apparently retroperitoneal primary GISTs. In Case 42, primary small intestinal *vs* colonic localization of the tumor could not be clearly established. Tumor size, available in all cases, varied from 1 to 36 cm (median 6.5 cm). Four small GISTs approximately 1 cm in diameter represented incidental findings during unrelated surgery ($n=2$), endoscopy ($n=1$) and autopsy ($n=1$).

In all, 29 GISTs had pure spindle cell morphology, 13 were epithelioid and eight revealed mixed histology with both spindle and epithelioid components. Pleomorphic features were seen in one spindle cell tumor. KIT expression was documented immunohistochemically in all analyzed cases. Representative histological and immunohistochemical images are shown in Figure 1. In all, 10

Table 3 Summary of clinicopathologic and molecular genetic data of 50 GISTs analyzed in this study

No	*Age*	*Sex*	*Location*	*Size (cm)*	*Mitosis/ 50HPF*	*Group*	*Histology*	*EM features*	*KIT/PDGFRA status*	*Frequency of LOH on 22q*	*Follow-up (months)*
1	78	F	S	1	0	1	Sp		KIT ex11 3′ITD	12.50%	DURC (24), IF gastric ca
2	76	F	S	1	0	1	Ep/Sp		WT	63.60%	DURC, IF autopsy
3	82	F	S	1	2	1	Sp		WT	No LOH	NA, IF gastritis endoscopy
4	69	M	S	1	0	1	Ep		PDGFRA ex18 PM	100%	NED (55), IF gastric ca
5	69	M	S	2	0	1	Sp		KIT ex11 DEL+PM	28.60%	NA
6	51	M	S	2.2	5	2	Sp	GANT	WT	22.20%	NED (33)
7	72	M	S	3	0	2	Sp		KIT ex11 PM	18.80%	NA
8	74	M	S	4	9	5	Sp		KIT ex11 DEL	71.40%	NED (4)
9	29	F	S	4.5	18	5	Sp/Pleo		WT	6.30%	MET disease (at operation)
10	75	F	S	4.5	10	5	Sp	GANT	KIT ex11 PM	No LOH	DURC, postoperation
11	62	M	S	4.5	7	5	Sp		KIT ex11 DEL	No LOH	NA
12	64	M	S	4.7	10	5	Sp/Ep		PDGFRA ex18 PM	No LOH	NA
13	40	M	S	5	0	2	Ep		PDGFRA ex18 PM	No LOH	NED (36)
14	33	M	S	5	1	2	Sp		KIT ex11 DEL	35.30%	NED (44)
15	37	F	S	5	5	2	Sp	GANT	KIT ex11 PM	6.30%	NED (50)
16	81	M	S	5.5	1	2	Sp		KIT ex11 DEL	21.40%	NED (194)
17	54	M	S	5.8	4	2	Sp		KIT ex11 PM	6.30%	NA
18	68	M	S	6	10	6	Sp		WT	100%	NA
19	82	M	S	6.5	5	3	Ep/Sp		PDGFRA ex18 PM	100%	NA
20	34	M	S	6.5	1	3	Sp		KIT ex11 DEL	31.30%	NED (32)
21	63	F	S	9	5	3	Sp/Ep		KIT ex11 PM	20.00%	NED (32)
22	67	F	S	9	2	3	Sp/Ep		KIT ex11 DEL	31.30%	NED (15)
23	67	M	S	9.5	8	6	Sp		KIT ex11 DEL+PM	78.60%	MET disease (NA)
24	29	M	S	10	2	3	Ep		KIT ex11 DEL	41.20%	NA
25	86	F	S	11	20	6	Ep	GANT	WT	5.90%	DOD (22)
26	37	M	S	12	100	6	Ep		KIT ex11 DEL+PM	94.10%	DOD (29)
27	49	F	S	16	1	3	Sp		KIT ex11 3′ITD	92.90%	NED (119)
28	60	M	S	16	35	6	Sp		KIT ex11 DEL	30.80%	NA
29	77	F	S	20	105	6	Ep	GANT	WT	100%	DOD (26)
30	49	M	S	27	5	3	Ep	GANT	PDGFRA ex18 PM	26.70%	NED (20)
31	60	M	S	30	85	6	Ep		KIT ex11 DEL+PM	No LOH	DOD (20)
32	63	F	SI	2	0	1	Sp/Ep		WT	7.70%	DURC (1), gastric ca
33	84	F	SI	2.1	0	2	Sp		WT	No LOH	NA
34	61	M	SI	4	5	2	Sp	GANT	KIT ex11 DEL	92.90%	NED (56)
35	43	F	SI	5	3	2	Sp		KIT ex11 DEL	11.80%	NED (56)
36	57	M	SI	6	1	3	Ep		WT	87.50%	NED (194)
37	64	F	SI	8	0	3	Sp/Ep		KIT ex17 PM	11.80%	NA
38	72	F	SI	9	1	3	Sp		WT	100%	NED (15)
39	61	M	SI	9	0	3	Sp		KIT ex11 DEL	44.40%	DUNK (312)
40	50	M	SI	30	16	6	Sp/Ep		KIT ex11 DEL	83.30%	DOD (54)
41	62	M	SI	36	30	6	Ep	GANT	KIT ex11 DEL	100%	DOD (30)
42	59	M	SI/C	18	0	3	Ep		KIT ex11 PM	75.00%	MET disease (48)
43	66	M	C	10	2	3	Ep		KIT ex11 PM	6.70%	DOD (31)
44	64	M	C	16.5	1	3	Sp		KIT ex11 PM	88.90%	DOD (8)
45	62	F	R	3.5	2	2	Sp	GANT	KIT ex11 DEL	18.20%	NA
46	63	M	R	7	13	6	Sp		KIT ex11 PM	100%	NED (27)
47	60	M	R	8.5	60	6	Sp		WT	No LOH	NED (9)
48	68	F	R	16	4	3	Sp		KIT ex9 INS	33.30%	DOD (45)
49	75	M	RP	13	10	6	Ep	GANT	PDGFRA ex18 PM	13.30%	DURC (29), CML
50	67	M	RP	21	75	6	Sp	GANT	KIT ex11 DEL+PM	No LOH	NED (56)

S: stomach; SI: small intestine; C: colon; R: rectum; Sp: spindle cell; Ep: epithelioid; Pleo: pleomorphic; RP: retroperitoneum; Ex: exon; DEL: deletion; ITD: internal tandem duplication; PM: point mutation; WT: wild type; NA: not available; NED: no evidence of disease; DOD: died of disease; DUNK: died of unknown causes; DURC: died of unrelated causes; IF: incidental founding; MET: metastatic.

previously reported GISTs[28] showed ultrastructural features suggestive of autonomic nerve differentiation and were considered gastrointestinal autonomic nerve tumor variants of GIST (GIST/GANT).

Based on tumor size and mitotic activity, GISTs were assigned to six clinicopathologic groups according to the expected biologic potential. A total of 17 cases were classified as benign or of very low malignant potential (groups 1 and 2), 15 of uncertain malignant potential (group 3) and 18 as malignant (groups 5 and 6).

Clinical follow-up data were available in 37 cases. In all, 19 patients showed no evidence of disease with an average follow-up of 55 months. Nine patients died of disease or developed intra-abdominal metastases. Five patients died of unrelated causes and one patient died of unknown causes 312 months after surgery.

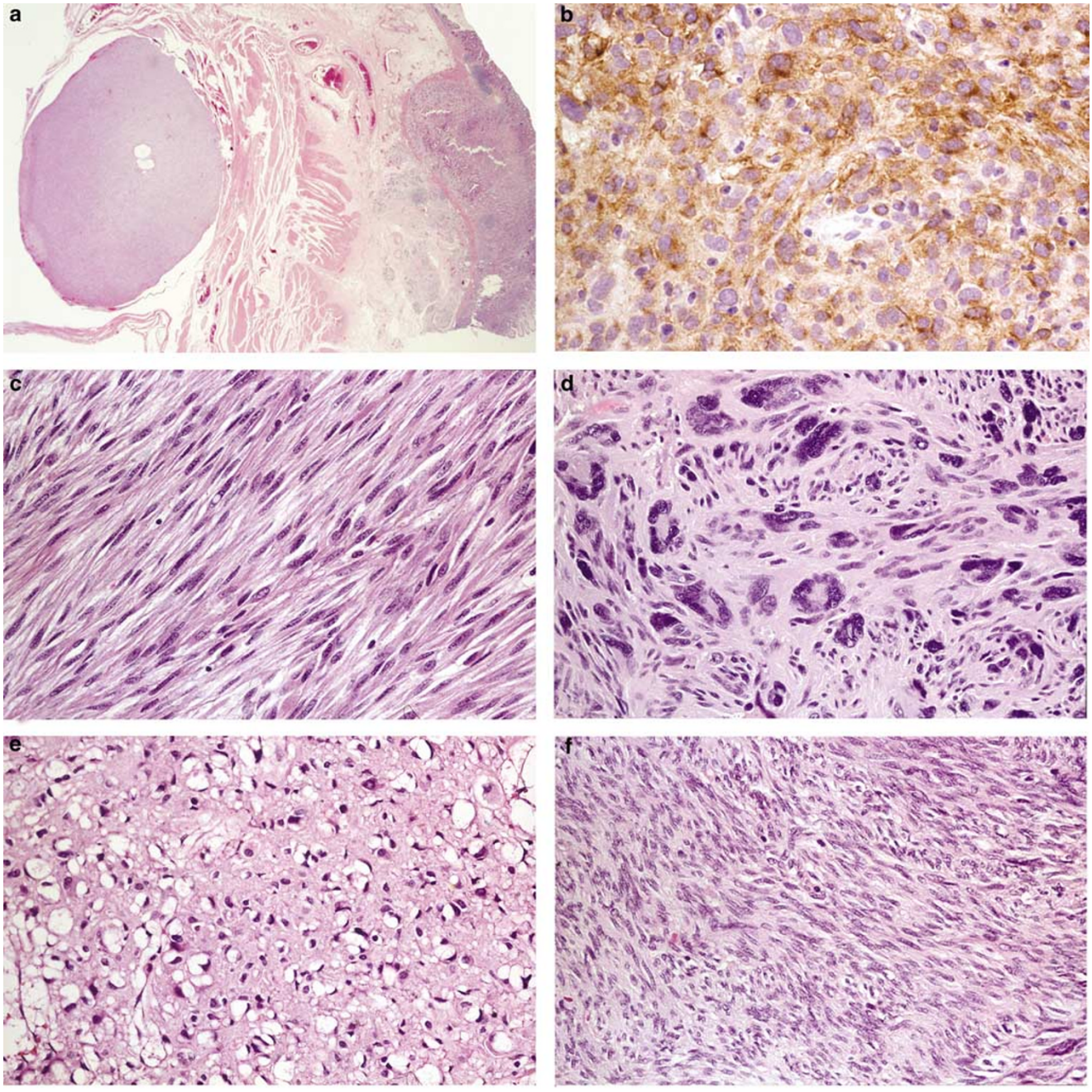

Figure 1 Representative histological and immunohistochemical images of GISTs analyzed in this study. Case 4, gastric carcinoma (**a**, right) and minimal GIST (**a**, left) with epithelioid histology and KIT expression (**b**) with documented LOH of all analyzed 22q markers; Case 32, spindle cell benign intestinal GIST (**c**) and Case 9, malignant gastric GIST with pleomorphic features (**d**), both tumors with documented isolated LOH at D22S425; Case 30, epithelioid gastric GIST/GANT (**e**) with isolated losses of D22S425 (IGLV locus) and D22S929 (NF2 locus); Case 41, spindle component of intestinal GIST/GANT (**f**) with documented LOH at all informative loci on chromosome 22q.

Genetic Studies

A total of 20 microsatellite markers were used to screen 50 tumors for LOH on chromosome 22q. Successful PCR amplification was obtained in 986 of 998 analyses (98.8%). LOH was calculated based on 754 heterozygous, informative markers and found in 295 analyses.

The pattern and frequency of LOH on chromosome 22q in 50 GISTs are shown in Figures 2 and 3. Representative examples of LOH analyses are shown in Figure 4.

In 15 of 50 (30%) analyzed tumors, more than 75% of informative markers were lost indicating loss of the entire of chromosome 22q, however, in nine (18%) cases LOH was not found at any of the

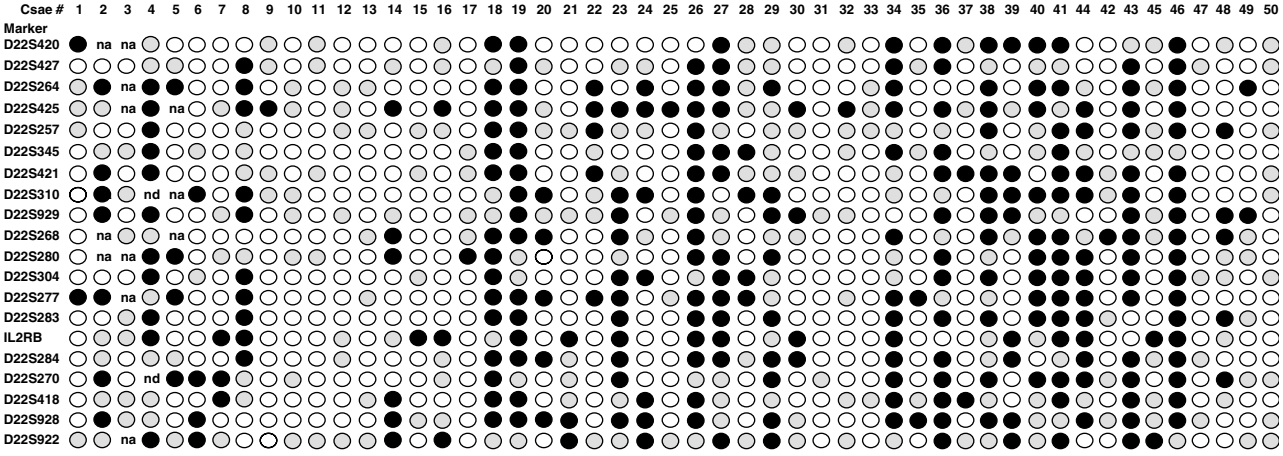

Figure 2 Patterns of LOH on chromosome 22q identified in 50 GISTs. Single assay is represented by a circle. White, black and gray colors indicate constitutional heterozygosity with retention of both alleles, loss of heterozygosity and constitutional homozygosity (noninformative loci), respectively. Markers are listed in consecutive order from the centromeric to the telomeric part of chromosome arm 22q. ua: unsuccessful PCR amplification; nd: not done.

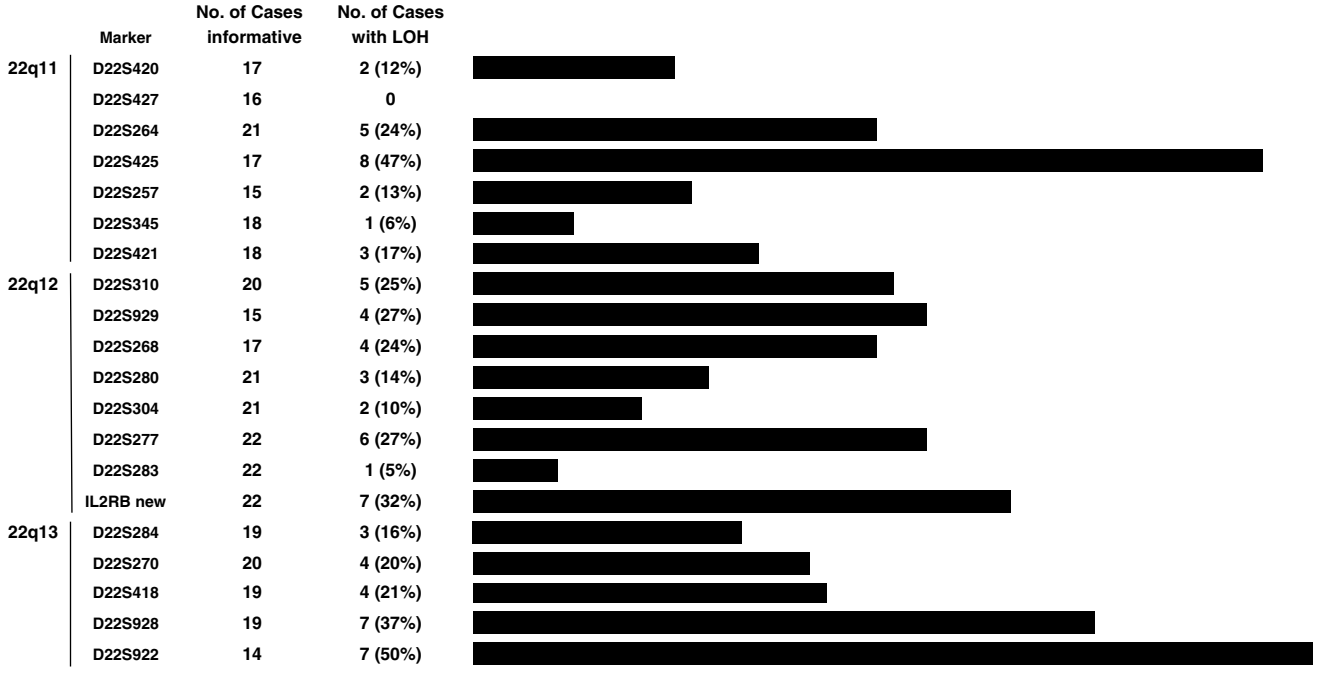

Figure 3 Frequencies of LOH at the 20 chromosome 22 loci examined in 50 GISTs.

analyzed loci. In the remaining 26 (52%) cases, isolated losses of one or multiple markers often clustering in the distinctive regions of chromosome 22q were found. The highest frequency of LOH was seen at D22S922 (50%) and at D22S425 (47%) mapped to 22q13.31 and 22q11.22, respectively. However, LOH of other markers including IL2RB, D22S277 and markers flanking or mapped to NF2 locus were also found in 20–30% of the informative cases.

In addition, five *NF2* intronic SNPs were analyzed in eight tumors noninformative for D22S929, the microsatellite marker mapped to the first *NF2* intron. However, finding of LOH in two of six tumors informative for at least one SNP marker did not substantially increase LOH frequency at *NF2* locus. The *NF2* coding sequences (exons 1–16) were evaluated for mutations in four GISTs. Two tumors with LOH at *NF2*, one with intragenic deletion documented by SNP analysis and one with LOH of marker mapped immediately distal to *NF2* locus, showed wild-type *NF2* sequences.

KIT and *PDGFRA* mutations were found in 39 of 50 (78%) analyzed tumors. *KIT* juxtamembrane

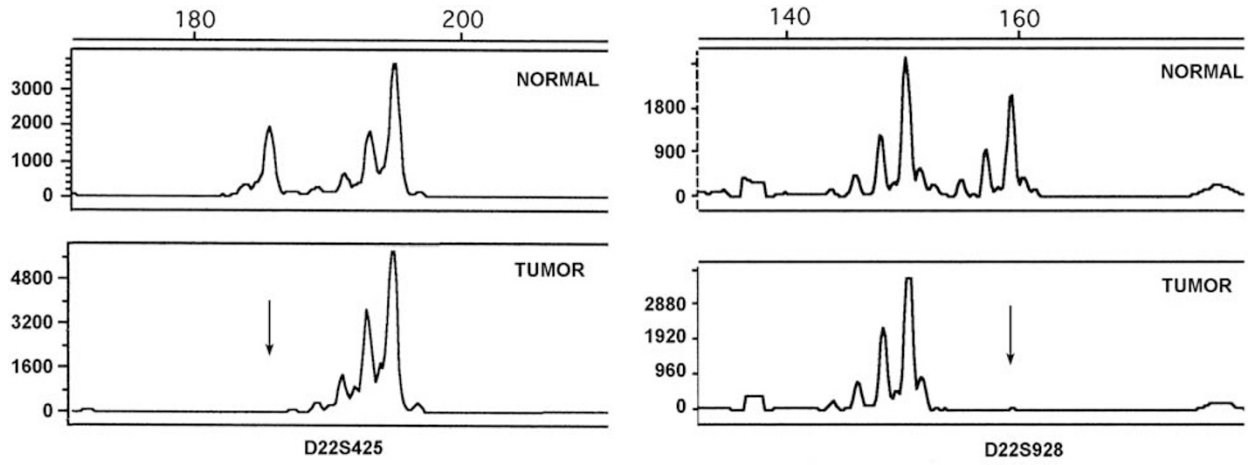

Figure 4 Two examples of LOH on chromosome 22 identified in GISTs by PCR amplification of D22S425 and D22S928 microsatellite markers and capillary gel electrophoresis. Black arrows indicate lost alleles.

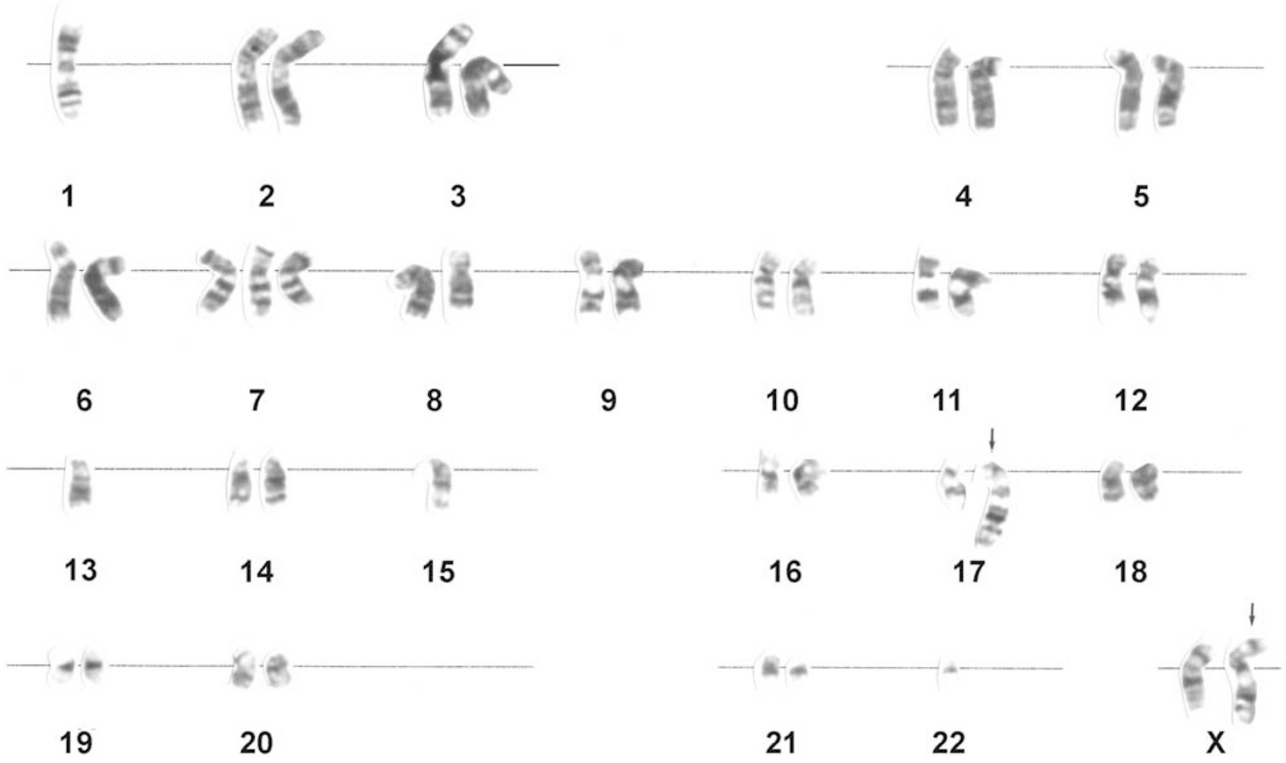

Figure 5 Karyotype 40–43,X, dic(X;1)(q12;p12),+7,−13,−15,der(17)t(1;17)(q23;q25),−22 identified in the Case 48, rectal malignant GIST.

domain (exon 11) mutations consisted of one to several codon deletions ($n=28$) sometimes complicated by coexisting point mutations (DEL/DEL+PM), single PMs ($n=9$) affecting codons 557, 559, 560 and 576 and in two cases internal tandem duplications (ITDs). In two tumors, respectively, a rare but typical AY502 ITD in *KIT* extracellular domain and N822 K PM in *KIT* tyrosine kinase domain (exon 17) were found. All six *PDGFRA* mutations affected codon 842 in tyrosine kinase domain leading to either valin (D842 V) or tyrosine (D842Y) substitution for aspartic acid. *KIT* and *PDGFRA* mutational status of all analyzed GISTs is shown in Table 3.

Cytogenetic study was performed in Case 48 and the following karyotype (Figure 5) was identified 40–43,X, dic(X;1)(q12;p12),+7,−13,−15,der(17)t(1;17)(q23;q25),−22 [cp14].

LOH on Chromosome 22q and Clinicopathological Features of GISTs

There were no substantial differences in LOH pattern and frequency among epithelioid and spindle cell tumors. GISTs assigned to groups 1 and 2 considered to be benign or of low malignant potential showed two times lower frequency of LOH on chromosome 22q than the highly malignant tumors asigned to group 6. Moreover, LOH of more than 75% of informative markers suggesting loss of the entire chromosome 22q were found in eight of 13 (61.5%) of GISTs assigned to group 6 compared with only two of 16 (12.5%) assigned to groups 1 and 2. However, isolated LOH at D22S425 mapped to 22q11.22 was equally found in both benign and malignant tumors. Intestinal GISTs revealed a higher LOH frequency (50.4%) than gastric (35.6%) tumors; however, GISTs assigned to groups 1 and 2 were two times more frequent among gastric (38.7%) than among intestinal (17.7%) tumors.

The patterns of LOH on chromosome 22q in GISTs/GANTs were similar to the ones seen in other GISTs. Three of 10 (33%) of these tumors showed loss of more than 75% of markers indicating possible loss of entire chromosome 22q. In five GISTs/GANTs, isolated LOH of single or multiple markers were found in the regions (including D22S425, NF2, IL2RB and 22q13.3) of chromosome 22q affected by deletions in other GISTs. In two GANTs, LOH was not found at any of the analyzed loci.

Three of four incidentally found, minimal GISTs (1 cm in diameter or less) showed LOH on chromosome 22q. In two tumors, all or the majority (63.3%) of informative markers were lost, indicating total or extended partial loss of chromosome 22q, respectively. In one minimal GIST, two isolated deletions were found. One of them involved D22S277 marker, shown in this study to be affected by LOH in 27% of all analyzed GISTs.

The patterns and frequencies of LOH on chromosome 22q were similar in the subcohorts of GISTs defined by KIT or PDGFRA mutational status.

Discussion

Gastrointestinal stromal tumors (GISTs) represent common mesenchymal tumors of the gastrointestinal tract driven by pathologic activation of KIT or PDGFRA tyrosine kinase receptors.[1] KIT and PDGFRA gain-of-function mutations are considered the early if not the first step in GISTs pathogenesis;[2,3] however, other genetic changes, mainly losses and gains have also been reported.[1] Losses of genetic material from chromosome 22 have been documented in GISTs by classical karyotyping, CGH, FISH and LOH studies.[3,7–16]

In this study, we have evaluated LOH on chromosome 22q in 50 well-characterized GISTs using 20 PCR-based microsatellite markers and capillary gel electrophoresis. In 15 cases (30%), LOH of all or almost all markers suggested loss of the entire chromosome 22. Previously published LOH and CGH studies on GISTs, respectively, found loss of the entire chromosome 22 in 39% and 26% of analyzed tumors.[9,15]

The frequency of loss of chromosome 22 in GISTs estimated by LOH or CGH studies differs substantially from the frequency of loss of chromosome 22 estimated by classical karyotyping. Two large, separate karyotyping studies of 19 and 52 GISTs reported loss of one or two copies of the chromosome 22, respectively, in 63 and 58% of analyzed cases.[3,14] The higher frequency of loss of chromosome 22 in GISTs detected by classic karyotyping compared to LOH or CGH studies could be an artifact related to the preferential culture of clones lacking chromosome 22. Recent report of primary GISTs with partial deletion of chromosome 22q and the recurrent tumor from the same patient with the loss of the entire chromosome 22 may support the notion of ongoing '*in vivo*' clonal evolution of GISTs.[16]

Higher frequency of partial *vs* total loss of chromosome 22 in GISTs analyzed by LOH or CGH could also be related to the occult retention of the parts of chromosome 22 due to submicroscopic translocations. Genetic material from chromosome 22 was recently shown to be a part of the marker chromosome in GIST karyotype with monosomy of chromosome 22.[29] In this study, discrepancy between karyotyping and LOH data was found in one analyzed tumor with the karyotype revealing monosomy of chromosome 22 and LOH analysis showing only partial loss of chromosome 22.

The highest frequency of LOH on chromosome 22q in GISTs was seen at D22S922 (50%), followed by D22S425 (47%) and D22S928 (37%) microsatellite markers. Two of three frequently deleted markers (D22S922, D22S928) are mapped to 22q13.3. This may suggest that deletion of 22q13.3 cytogenetic region plays a role in GISTs development. Recently published GIST karyotype with the specific deletion of 22q13 could partially support this notion[3] as well as a study on GISTs/GANTs that reported exclusive loss of chromosome 22q13 cytogenetic region in one of five analyzed cases.[16] Previous LOH studies on GISTS did not report significant LOH at 22q13.3;[11,12,15] however, only one 22q13.3 microsatellite marker, D22S274, positioned proximal to D22S928, was evaluated and found to be deleted in some cases.[11] Also in this study, markers mapped proximal to D22S274 at 22q13.2 were not commonly deleted. This again supports the mapping of the common deletion region in GISTs to 22q13.3–qter. However, further studies are required to define minimal regions of deletions and identified putative tumor suppressor gene/genes inactivated in GISTs. The cytogenetic region 22q13.3 has been shown to be affected by deletions in a spectrum of human cancers including breast,

colon, ovarian and oral carcinomas as well as actrocytomas.[30–33]

D22S425, mapped to 22q11.22 cytogenetic region, was the second most frequently lost marker in GISTs. 22q11.22 was not indicated as a common region of deletion in previously published GIST LOH studies.[11,15] However, LOH at D22S446, which currently is mapped relatively closed to D22S425 was found in 53.8% of analyzed GISTs.[15] Considering the updated mapping of the markers (Table 2) and our and previously published data,[15] the second most common region of deletion on chromosome 22q in GISTs might be defined by LOH at D22S425 and D22446. This region includes immunoglobulin lambda light chain variable region (IGLV) genes and other genes whose functions are not well known (www.ncbi.nlm.nih.gov).

Frequent LOH at NF2 locus (53%) was reported by Fukosawa *et al*[11] in the first comprehensive study of LOH in GISTs and recently confirmed by Pylkkanen *et al*.[15] In our study, LOH at NF2 locus was seen only in 27% of analyzed tumors, almost two times less than LOH at D22S922, the most commonly deleted marker. Although in some cases, *NF2* was affected by an intragenic deletion as shown using the panel of SNP markers, no mutations were found in four GISTs with documented LOH at NF2 locus or its vicinity. Fukosawa *et al*[11] reported one loss-of-function and one splicing mutation in 22 analyzed GISTs (9%); however, another study failed to find *NF2* mutations in five primary GISTs.[15] Considering all this, it appears that 'Knudson type' two hit inactivation of NF2 cannot be confirmed in GISTs.

An increased frequency of LOH was also seen at Interleukin 2 receptor beta (IL2RB) locus and D22S277 microsatellite marker, mapped to 22q12.3 cytogenetic region. This finding may correspond to previously reported increased frequency of LOH in the region defined by D22S683 and D22S445 microsatellite markers.[15] This region overlaps with the common region of deletions reported in astrocytomas[34] and breast carcinomas.[35]

Loss of genetic material from chromosome 22 was documented in benign and malignant, sometimes called low- and high-risk, GISTs.[9,11] Although some of the studies linked LOH on chromosome 22q to tumor progression and unfavorable outcome,[12,15] in our study, loss of genetic material from chromosome 22 was documented in three of four minimal GISTs incidentally detected due to other medical procedures. This suggests that LOH on chromosome 22 or loss of chromosome 22 occurs early at the tumor formation. However, frequency of LOH in malignant GISTs assigned to group 6 (>5 cm, >5 mitosis/50HPF) was higher than in benign tumors assigned to groups 1 and 2 (≤ 2 cm, <5 mitosis/50HPF and >2 cm <5 cm, ≤ 5 mitosis/50HPF). Of 13 tumors assigned to group 6, eight (61.5%) revealed deletions of at least 75% of chromosome 22q markers, suggesting loss of the entire chromosome 22. In contrast, only two of 16 (12.5%) tumors assigned to groups 1 and 2 with usually favorable outcome showed extensive losses on chromosome 22q. This may suggest that initial LOH on chromosome 22 at the early stage of tumor formation is followed by either accumulation of LOH on chromosome 22 due to genetic instability or ongoing clonal selection, giving growing advantage to the tumor cells with losses of chromosome 22.

In this study, isolated losses at IGLV locus (D22S425) were found in both benign and malignant tumors. The LOH studies on astrocytomas and breast carcinomas reported the same type of isolated losses on chromosome 22q in both early lesions and highest malignancy grade tumors. However, significant increase of LOH frequency was seen only in the latter.[30,33,34] More tumors with isolated losses of specific chromosome 22q markers should be studied to confirm this phenomenon in GISTs.

Intestinal GISTs showed higher frequency of LOH on chromosome 22q than in gastric tumors. However, this may be related to the higher number of benign, group 1 and 2 tumors in the latter cohort. There was no significant difference in the LOH pattern on chromosome 22q between GISTs defined by morphological (epithelioid *vs* spindle cell) and molecular genetic features (KIT mutant *vs* PDGFRA mutant *vs* KIT and PDGFRA wild type).

GI autonomic nerve tumors (GANTs), which show ultrastructural features suggestive of autonomic nerve differentiation, are now considered GIST variants based on KIT expression and presence of GIST-specific KIT[28] or PDGFRA-activating mutations. Recent FISH/CGH-based study reported loss of whole or part of chromosome 22q with the common overlapping area at 22q13 in five malignant intestinal GISTs/GANTs.[16] It was also speculated that deletion of 22q13 may be a characteristic genetic feature of GANTs.[16] In this study, we have evaluated 10 previously reported GANTs for the LOH on chromosome 22. The pattern of detected losses was similar to the one seen in other GISTs, giving no indication that GANTs would be distinctive in their involvement of chromosome 22.

In summary, we have shown that LOH on chromosome 22q in GISTs could be found at the early tumor formation and late stage of tumor progression. Although similar LOH patterns were seen in both early and late stages of tumor development, the frequency of total losses significantly increased in highly malignant tumors. Based on this study, at least three different regions harboring genes important for GISTs development can be defined and should be a subject of further molecular genetic studies.

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