COLORECTAL CANCER

Epithelial and stromal genetic instability contributes to genesis of colorectal adenomas

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Background: Previously, we indicated that stromal genetic instability might contribute to tumorigenesis of both sporadic and ulcerative colitis associated colorectal adenocarcinomas. Considering the established adenoma-adenocarcinoma sequence, in this study we analysed genetic instability in colorectal adenoma cells and surrounding stroma.

Methods: In 164 colorectal tumours (34 hyperplastic polyps, 38 tubular adenomas with low grade dysplasia (TA-L), 51 tubular adenomas with high grade dysplasia (TA-H), and 41 invasive carcinomas), epithelial and stromal genetic instability with National Cancer Institute standard microsatellite markers and chromosome 17 (Chr17) markers, were analysed by a combination of laser capture microdissection and GeneScan approaches.

Results: While frequencies of both loss of heterozygosity (LOH) and microsatellite instability (MSI) were extremely low in hyperplastic polyps, LOH in tubular adenomas was detected in both epithelial (TA-L 13.2%, TA-H 27.5%) and stromal (5.3% and 5.9%, respectively) elements, along with MSI (5.3% and 13.7%, and 5.3 and 5.9%, respectively). Frequencies of epithelial alterations were higher in TA-H than in TA-L, and greatest in the carcinoma group. On the other hand, frequencies of stromal LOH or MSI were almost constant (5.3% ~ 17.1%, 5.3% ~ 17.1%, respectively) in adenomas and invasive carcinomas. In addition, p53 was found to be significantly overexpressed in a greater proportion of TA-L with LOH than in those without genetic instability.

Conclusion: The results indicate the presence of genetic alterations in stroma from an early stage of carcinogenesis, accompanied by stepwise increasing genetic instability of epithelia with progression to cancer. Thus microenvironmental changes due to genetic alteration in Chr17 markers in stromal cells may play an important role in colon adenoma and adenocarcinoma development.

Interactions between epithelial and mesenchymal cells in various organs play important roles in development,^{1,2} differentiation,^{3,4} and growth.^{3,4} However, the majority of studies on colorectal cancer have focused on epithelial carcinoma cells and any contribution of mesenchymal cells to carcinogenesis has received only limited attention. Analysis of surrounding stromal cells should be conducted for a more comprehensive understanding.

It is widely accepted that accumulation of genetic alterations is involved in transition from the normal mucosa to adenocarcinomas via adenomas in the colorectum. *APC* mutation and loss, *K-ras* mutation, *Smad2/4* loss, and *TP53* mutation and loss are well documented,⁵⁻⁸ along with altered DNA methylation with progression to carcinoma.⁹ In colorectal carcinomas, two types of genetic instability, microsatellite instability (MSI), and chromosomal instability (CIN) are frequently detected. MSI is characterised by accumulation of somatic alterations in simple repeated nucleotide sequences called microsatellites^{10,11} while CIN is characterised by loss of heterozygosity (LOH) at sites of a number of cancer related genes.^{12,13} The precise mechanisms leading to CIN are not well understood. In sporadic colorectal carcinomas (S-CRC), it has been reported that approximately 15% of tumours demonstrate MSI,¹⁴ approximately 50% exhibiting LOH.¹⁵

Previously, using a combination of microdissection and genetic instability analysis with microsatellite markers recommended by the National Cancer Institute (NCI), as well as others close to the *p53* and *BRCA1* genes on chromosome 17 (Chr17), we revealed that not only epithelial but also stromal elements demonstrate genetic instability in invasive colorectal carcinomas and that such stromal

alteration might influence the genesis of S-CRCs.¹⁶ In addition, Chr17 markers were found to be more sensitive than NCI markers, especially for detecting stromal genetic instability. The adenoma-carcinoma sequence is accepted as playing a major role in colorectal carcinogenesis, and colorectal adenomas can be regarded as precancerous.⁶ In addition, MSI was reported to be present in 13% of colorectal tubular adenomas, 53% of serrated adenomas, and 27% of hyperplastic polyps in one series.¹⁷

In the present study, using a laser capture microdissection method, we examined genetic instability in both epithelial and stromal cells of tubular adenomas in the colorectum, and analysed possible correlations with histological progression. For this purpose, a combination of Chr17 and NCI standard microsatellite markers was applied to increase sensitivity.

MATERIALS AND METHODS

Samples

A total of 123 sporadic colorectal polyps and 41 sporadic colorectal invasive carcinomas were examined in this study. Specimens were obtained by either endoscopic polypectomy or surgical resection. More than two expert pathologists histologically diagnosed the hyperplastic polyps (HP), tubular adenomas with low grade (TA-L) or high grade (TA-H)

Abbreviations: Ca, adenocarcinoma; Chr17, chromosome 17; CIN, chromosomal instability; HP, hyperplastic polyp; LI, labelling index; LOH, loss of heterozygosity; MSI, microsatellite instability; MSI-H, high level MSI; MSI-L, low level MSI; NCI, National Cancer Institute; PCR, polymerase chain reaction; S-CRC, sporadic colorectal carcinoma; TA-H, tubular adenoma with high grade dysplasia; TA-L, tubular adenoma with low grade dysplasia

Figure 1 (A) Representative results of laser capture microdissection analysis and GeneScan (MYCL) examination of cancer tissue. After a tumour lesion was confirmed by examination of haematoxylin-eosin stained sections, tumour epithelium and surrounding stromal cells were dissected from serial sections of paraffin embedded tissue. Original magnification: $\times 40$. (B) Representative GeneScan findings for epithelium (E) and stroma (S) from adenomas. N, Normal epithelium or stroma.

dysplasia, and adenocarcinomas (Ca) independently.¹⁸ Samples of histologically normal mucosa from each patient were obtained for comparison as controls for the genetic instability analysis. Additional controls were provided by 10 normal mucosa specimens from colorectums without tumours surgically resected from diverticulosis patients. Tissues were formalin fixed and paraffin embedded, and serial 3 μ m thick sections were cut for haematoxylin-eosin staining and immunohistochemistry, along with serial 10 μ m thick sections for microdissection. For the latter, deparaffinised sections were used after nuclear staining

(Histogene LCM Frozen Section Staining Solution; Arcturus, Mountain View, California, USA).

DNA extraction

Microdissection and DNA extraction were carried out as described previously.¹⁶⁻¹⁹ Briefly, epithelial and adjacent stroma tissues in the lamina propria were carefully microdissected with a laser capture microdissection system (LM200 and Pixcell IIe; Arcturus) taking care to avoid cross contamination. Normal control DNA for each case was obtained from histologically normal mucosa sufficiently

Table 1 Clinicopathological features of colorectal tumours

Clinicopathological factor	Normal control (%)	HP (%)	TA-L (%)	TA-H (%)	Ca (%)
Total No	10	34	38	51	41
Age (y)*	51.5 (12.6)	61.0 (12.6)	68.8 (10.5)	66.4 (10.4)	68.2 (9.9)
Tumour size (mm)*		5.7 (3.0)	6.1 (4.4)	10.0 (5.9)	41.4 (15.9)
Sex					
Male	7 (70.0)	25 (73.5)	28 (73.7)	39 (76.5)	28 (68.3)
Female	3 (30.0)	9 (26.5)	10 (26.3)	12 (23.5)	13 (31.7)
Location					
Proximal	5 (50.0)	10 (29.4)	21 (55.3)	19 (37.3)	13 (31.7)
Distal	5 (50.0)	24 (70.6)	17 (44.7)	32 (62.8)	28 (68.3)
Differentiation					
Well					18 (43.9)
Moderate					20 (48.8)
Poor					3 (7.3)

*Values are mean (SD).

HP, hyperplastic polyp; TA-L, tubular adenoma with low grade dysplasia; TA-H, tubular adenoma with high grade dysplasia; Ca, adenocarcinoma.

distant from the tumour. The resulting DNA samples were incubated overnight at 65°C in lysis buffer containing proteinase K (PicoPure DNA extraction kit; Arcturus) and applied for polymerase chain reaction (PCR) analysis after heat inactivation of proteinase K at 95°C for 10 minutes.

Microsatellite analysis

PCR was performed for four microsatellite markers selected for analysing allelic instability on Chr17 (dinucleotide repeats), D17S796, TP53, D17S786 (close to the p53 gene),²⁰ and D17S579 (close to BRCA1),²¹ along with five NCI standard markers for S-CRC analysis, BAT25, BAT26 (mononucleotide repeats), D2S123, D3S346, and D17S250 (dinucleotide repeats),²² and in chromosome 1, MYCL (tetranucleotide repeat),²³ using fluorescent primer pairs (Applied Biosystems, Foster City, California, USA). Genomic instability was detected with the aid of an ABI Prism 310 or ABI Prism 3100 *Avant* Genetic Analyser, and analysed with GeneScan Analysis and Genotyper software (version 3.7; PE Applied Biosystems). MSI or LOH positivity was determined compared with control DNA (fig 1B).

Immunohistochemistry

Monoclonal anti-p53 (DO7, ×300 dilution; Novocastra Lab, Newcastle, UK) and polyclonal anti-Ki67 (MIB1, ×100

dilution, Dako, Glostrup, Denmark) antibodies were used for immunohistochemistry. Deparaffinised histological sections (3 µm thick) from paraffin embedded tissue specimens and frozen sections were cut for immunohistochemistry, conducted using the streptavidin-biotin peroxidase complex method. LSAB2 (DakoCytomation, Glostrup, Denmark) and Envision Plus (DakoCytomation) kits were applied with slight modification in our laboratory,²⁴ after microwave oven heating (500 W for five minutes, three times, in 10 mM citrate buffer, pH 6.0) to retrieve antigenic activity. Endogenous peroxidase was inhibited by incubation with 0.3% H₂O₂ in methanol for 30 minutes. Then, histological sections were incubated with the first antibodies overnight at 4°C and then the second antibodies and substrate. Faint nuclear counterstaining was finally achieved with 5% methyl green. Labelling indices (LI) for p53 and Ki-67 were calculated as percentages of nuclear positive cells in 1000 cells counted.

Statistical analysis

Differences in distributions between variables were calculated using the χ^2 test or Fisher's exact test, as appropriate. Probability values <0.05 were considered significant.

Table 2 Genetic instability of the epithelium and stroma in colorectal tumours

		MSI+/informative cases (%)*			LOH+/informative cases (%)*		
		Epithelium	Stroma	p Value†	Epithelium	Stroma	p Value†
NCI markers	Control	0/10 (0)	0/10 (0)	NS	0/10 (0)	0/10 (0)	NS
	HP	1/34 (2.9) ^a	1/34 (2.9)	NS	0/34 (0) ^{j,k}	0/34 (0)	NS
	TA-L	2/38 (5.3) ^b	0/38 (0)	NS	3/38 (7.9) ^l	0/38 (0) ^w	NS
	TA-H	2/51 (3.9) ^c	1/51 (2.0)	NS	6/51 (11.8) ^k	1/51 (2.0)	NS
	CA	9/41 (22.0) ^{a,b,c}	1/41 (2.4)	0.0069	10/41 (24.4) ^{j,k}	4/41 (9.8) ^w	NS
Chr17 markers	Control	0/10 (0)	0/10 (0)	NS	0/10 (0) ^m	0/10 (0)	NS
	HP	1/34 (2.9) ^d	0/34 (0)	NS	1/34 (2.9) ⁿ	1/34 (2.9)	NS
	TA-L	1/38 (2.6) ^e	0/38 (0)	NS	1/38 (2.6) ^{o,p}	2/38 (5.3)	NS
	TA-H	5/51 (9.8)	2/51 (3.9)	NS	9/51 (17.6) ^{n,p,q}	3/51 (5.9)	NS
	CA	8/41 (19.5) ^{d,e}	3/41 (7.3)	NS	21/41 (51.2) ^{m,o,q}	4/41 (9.8)	<0.0001
Total markers‡	Control	0/10 (0) ^f	0/10 (0)	NS	0/10 (0) ^r	0/10 (0)	NS
	HP	3/34 (8.8) ^a	1/34 (2.9)	NS	1/34 (2.9) ^{s,t}	1/34 (2.9) ^x	NS
	TA-L	2/38 (5.3) ^h	2/38 (5.3)	NS	5/38 (13.2) ^u	2/38 (5.3)	NS
	TA-H	7/51 (13.7) ⁱ	3/51 (5.9)	NS	14/51 (27.5) ^{t,v}	3/51 (5.9)	0.0035
	CA	14/41 (34.2) ^{f,g,h,i}	3/41 (7.3)	0.0027	25/41 (61.0) ^{r,s,u,v}	7/41 (17.1) ^x	<0.0001

*Percentage microsatellite instability (MSI) or loss of heterozygosity (LOH)/informative case.

†Significant difference between epithelium and stroma.

‡Total markers: National Cancer Institute (NCI) markers, chromosome 17 (Chr17) markers, and MYCL.

HP, hyperplastic polyp; TA-L, tubular adenoma with low grade dysplasia; TA-H, tubular adenoma with high grade dysplasia; Ca, adenocarcinoma.

a, b, d, e, f, i, k, l, n, p, w, x, p<0.05; c, g, p<0.01; h, j, m, t, v, p<0.005; q, r, p<0.001; s, u, p<0.0001.

Figure 2 Frequencies of genetic instability in colorectal tumours. C, normal mucosa; HP, hyperplastic polyp; TA-L, tubular adenoma with low grade dysplasia; TA-H, tubular adenoma with high grade dysplasia; Ca, invasive adenocarcinoma; NCI, National Cancer Institute standard markers; Chr17, chromosome 17 markers. (A) Epithelial loss of heterozygosity (LOH) with NCI and Chr17 markers. (B) Epithelial microsatellite instability (MSI) with NCI and Chr17 markers. (C) Stromal LOH with NCI and Chr17 markers. (D) Stromal MSI with NCI and Chr17 markers. (E) Epithelial LOH and MSI with all markers in combination (NCI and Chr17 markers and MYCL). (F) Stromal LOH and MSI with all markers in combination. Percentages of MSI or LOH positive/informative lesions (%) are shown by the vertical axis. Bars indicate positive for 1, 2, 3, or 4 or more markers. * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.005$, § $p < 0.001$, ¶ $p < 0.0001$. a, b, d, i, m, p, $p < 0.05$; g, l, $p < 0.01$; f, h, $p < 0.005$; °, $p < 0.001$; c, e, n, $p < 0.0001$.

Ethics

This work using pathological samples in Kitasato University and Kitasato University East Hospitals was approved by our Medical School and University Hospital Ethics Committee.

RESULTS

Clinicopathological findings for the colorectal lesions are summarised in table 1. Results for genetic instability are

summarised in table 2. No genetic instability was detected in control mucosa specimens.

Genetic instability in tumour epithelium (table 2, fig 2A, B)

Frequencies of epithelial LOH with NCI markers were increased stepwise from HP 0%; TA-L 7.9%; TA-H 11.8%; and Ca 24.4% (table 2). However, with the Chr17 markers,

Figure 3 Representative immunohistochemical staining of p53 and Ki-67. p53 in hyperplastic polyps (HP) (A), in tubular adenomas with low grade dysplasia (TA-L) (B), in tubular adenomas with high grade dysplasia (TA-H) (C), and in adenocarcinomas (D). Ki-67 in HP (E), in TA-L (F), in TA-H (G), and in Ca (H). Original magnification: $\times 100$.

frequencies were significantly lower in HP and TA-L than in TA-H ($p < 0.05$) (fig 2A). LOH in Ca was significantly increased compared with HP and TA-L using both NCI (HP $p < 0.005$, TA-L $p < 0.05$) and Chr17 (HP $p < 0.0001$, TA-L $p < 0.0001$) markers and compared with TA-H with Chr17 markers ($p < 0.01$) (fig 2A). Considering the numbers of positive markers of LOH, only one was positive with the NCI recommended examples in each lesion, while multiple Chr17 markers showed LOH in TA-H and Ca (fig 2A). The frequencies of LOH with Chr17 markers were significantly greater than NCI markers, particularly for two or more

markers (TA-H, $p < 0.01$; Ca, $p < 0.0001$) and three or more markers (TA-H $p < 0.05$; Ca $p < 0.005$) (fig 2A).

Regarding epithelial MSI, the frequencies with NCI markers were significantly higher for Ca than HP and TA-L categories (HP $p < 0.05$, TA-L $p < 0.05$).

Genetic instability in tumour stroma (table 2, fig 2C, D)

Frequencies of stromal LOH with NCI markers were 2.0% in TA-H, 9.8% in Ca, but none in HP and TA-L. The Ca value was significantly greater than that for TA-L ($p < 0.05$) (fig 2C).

Table 3 Relations between genetic instability and tumour location

			LOH+		MSI+		LOH+ and MSI+		
			Total	Epithelium	Stroma	Epithelium	Stroma	Epithelium	Stroma
HP	Proximal	10	1/10	0/10	2/10	0/10	0/10	1/10	
	Distal	24	0/24	0/24	1/24	0/24	0/24	0/24	
TA-L	Proximal	21	2/21	2/21	2/21	0/21	0/21	0/21	
	Distal	17	3/17	0/17	0/17	2/17	0/17	0/17	
TA-H	Proximal	19	4/19	2/19	3/19	2/19	2/19	0/19	
	Distal	32	8/32	1/32	2/32	1/32	0/32	0/32	
Ca	Proximal	13	0/13*	3/13	5/13†	0/13	2/13	1/13	
	Distal	28	16/28*	2/28	0/28†	1/28	7/28	1/28	

Proximal: caecum, ascending colon, and transverse colon.

Distal: descending colon, sigmoid colon, and rectum.

HP, hyperplastic polyp; TA-L, tubular adenoma with low grade dysplasia; TA-H, tubular adenoma with high grade dysplasia; Ca, adenocarcinoma.

* $p < 0.05$; † $p < 0.005$.

Frequencies of LOH with Chr17 markers gradually increased with progression but without significance (2.9% in HP, 5.3% in TA-L, 5.9% in TA-H, and 9.8% in Ca). Frequencies of MSI were very low (fig 3D).

Genetic instability with all markers in combination (table 2, fig 2E, F)

With the total of 10 NCI, Chr17, and MYCL markers, frequencies of epithelial LOH tended to be increased with histological progression. Considering the numbers of positive markers, epithelial LOH was detected with multiple markers significantly more frequently in the TA-H and Ca categories (fig 2E).

Frequencies of epithelial MSI with all markers in combination also tended to increase along with histological progression. Values were significantly higher for Ca than HP ($p < 0.01$), TA-L ($p < 0.005$), or TA-H ($p < 0.05$). Considering the numbers of positive markers, LOH was detected with multiple markers in TA-H and Ca, and MSI was detected with multiple markers in TA-L, TA-H, and Ca. Although stromal genetic instability was infrequent, a significant difference was found between Ca and HP LOH ($p < 0.05$).

Relations between status for genetic instability and tumour location (table 3)

Data for genetic instability with reference to tumour location are summarised in table 3. There was no significant link for HP, TA-L, or TA-H. However, positivity for MSI in Ca was significantly higher ($p < 0.005$) on the proximal side than on the distal side. Furthermore, the frequency of epithelial LOH

in Ca of the distal colon was significantly higher ($p < 0.005$) than that on the proximal side. No significant correlations between stromal genetic instability and tumour location were found.

Relation between p53 overexpression, cell proliferation activity (Ki-67 LI), and epithelial genetic instability

As the Chr17 markers are in the vicinity of the p53 gene, the relation between epithelial genetic instability and p53 protein expression was examined by immunohistochemistry, representative findings being shown in fig 3. In HP and TA-H, p53 protein expression was significantly greater in LOH positive lesions than in those without genetic instability (HP $p < 0.005$, TA-H $p < 0.005$) (fig 4A), but no variation in cell proliferation, as assessed by Ki-67, was apparent (fig 4B). No significant correlation between MSI positivity and p53 overexpression was found.

DISCUSSION

Participation of genetic instability in colorectal carcinoma development has been indicated by many reports of LOH and MSI.²⁵⁻²⁶ The fact that MSI exists in 10% of colorectal adenomas²⁷ may imply a role in the adenoma-carcinoma sequence and the present study provided concrete evidence that this is indeed the case. Previously, we revealed that not only epithelium but also stroma may demonstrate genetic instability in sporadic invasive colorectal carcinomas.¹⁶ Furthermore, in an ulcerative colitis associated tumour series, representative of the established chronic inflammation-carcinoma sequence,²⁸⁻²⁹

Figure 4 (A) p53 overexpression with reference to the genetic instability status of colorectal tumour epithelium. (B) Ki-67 expression with reference to the genetic instability status of colorectal tumour epithelium. HP, hyperplastic polyp; TA-L, tubular adenoma with low grade dysplasia; TA-H, tubular adenoma with high grade dysplasia; Ca, adenocarcinoma. LOH, loss of heterozygosity; MSI, microsatellite instability. Values are mean (SD).

we found both epithelial and stromal genetic instability in regenerative mucosa, dysplasia, and adenocarcinoma, suggesting an influence of the stromal microenvironment on epithelial cells from an early stage in the development of neoplasms.¹⁹

In the present study, we further demonstrated genetic instability in epithelial and stromal cells, and analysed their correlation with histological progression of colorectal tumours. In a previous report,¹⁷ the prevalence of epithelial genetic instability varied between tubular adenoma (MSI in 13%) and serrated adenoma (MSI in 57%). Therefore, we focused on tubular adenomas because villous and serrated adenomas may possess different biological and genetic features.⁹ We found that epithelial genetic instability increases with progression of histological lesions, whereas LOH and MSI in stromal elements tend to be more constant in TA-L, TA-H, and invasive carcinomas. In line with the finding of frequent genetic changes in mammary stromal tissue in breast carcinoma patients,³⁰ and the demonstration of conversion of colonic adenomas to adenocarcinomas in nude mice with inflammation associated stroma,²⁷ the results of this study suggest that early changes in the stroma may be associated with epithelial tumorigenesis. When MSI was categorised into high level (MSI-H; MSI found in four or more markers) and low level (MSI-L; MSI found in 1–3 markers) MSI, MSI-H was found to exist in TA-H and Ca epithelium (fig 2E) and not in stroma (fig 2F). Although MSI-H, defects of mismatch repair enzymes, and MSI in mononucleotide markers such as BAT-25 or BAT-26 are considered to be correlated,^{25–26} some stromal lesions with MSI-L showed major shift bands in mononucleotide markers (fig 1B, BAT-26). The relations between the major shift bands in stroma and mismatch repair enzymes remain to be analysed. Thus the significance of MSI-L found in stroma should be clarified in relation to tumorigenesis.

Previously, we reported that cell proliferation estimated by Ki-67 LI increased stepwise from TA-L and TA-H through to invasive carcinomas.³¹ In this study, however, we did not find significant differences in Ki-67 LI among lesions with or without genetic instability. Furthermore, our immunohistochemical investigation showed p53 overexpression was significantly greater in LOH positive than LOH negative TA-H. Some cases of TA-H showed diffuse p53 staining which might indicate a mutation in the tumour suppressor gene although we could not confirm this here. However, other TA-H showed scattered expression of p53 with a higher LI than TA-L (data not shown). Therefore, the increased p53 LI in TA-H with LOH may be due to the coexistence of wild-type and mutant p53 components.

Previously, evidence was presented that MSI-H colorectal tumours are more likely to be right sided (proximal) than their MSI-L or MSI stable counterparts.^{14–32} The present study also demonstrated epithelial MSI predominantly in carcinomas of the proximal colon. In addition, all epithelial LOH positive carcinomas were located in the distal colon, suggesting differences in carcinogenesis between the two sites.³³ To our knowledge, there have hitherto been no reports of significant correlations between LOH and tumour location. As the number of tumours analysed was rather small in the present study, this point needs further investigation with a larger series.

In conclusion, the present results suggest that stromal genetic instability is associated with colorectal adenoma development. Contrary to the general belief that abnormalities in stroma occur as reactions to epithelial tumour cells, our data support the hypothesis that an alternative pathway with prior influence of stromal genetic instability on epithelial cells may exist in the adenoma-adenocarcinoma sequence. Further studies are warranted to test this hypothesis.

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EDITOR'S QUIZ: GI SNAPSHOT

Copious amounts of ascites

Robin Spiller, *Editor*

Clinical presentation

An 81 year old woman consulted a local clinic with a three month history of progressive abdominal distension and subsequent diarrhoea, and appetite loss for one month. She was referred to our hospital for further evaluation.

On physical examination there were no signs of peritonitis or pancreatitis. Laboratory examination revealed slight anaemia (haemoglobin 10.8 g/dl) and elevation of a tumour marker (carcinoembryonic antigen 21 ng/ml). Sonography showed non-mobile ascitic fluid with internal irregular echogenic structures throughout the whole abdominal cavity (fig 1). Ascites with attenuation slightly higher than water was demonstrated on computerised tomography but the heterogeneity seen on sonography was not obvious (fig 2).

Question

What is the diagnosis?

See page 714 for answer

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Figure 1 Sonography showing non-mobile ascitic fluid with internal irregular echogenic structures throughout the whole abdominal cavity.

Figure 2 Computerised tomography showing ascites with attenuation slightly higher than water, but the heterogeneity seen on sonography was not obvious.