British Journal of Cancer (1995) 72, 376-382

Microsatellite instability in ovarian neoplasms

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Summary Microsatellite instability has been observed in a variety of sporadic malignancies, but its existence in sporadic ovarian cancer has been the subject of conflicting reports. We have performed a polymerase chain reaction-based microsatellite analysis of DNAs extracted from the neoplastic and non-neoplastic tissues of 41 ovarian cancer patients. Tumour-associated alterations were observed in seven (17%) of these cases. Clinicopathological correlations revealed that: (1) alterations among tumours classified as serous adenocarcinomas occurred with relatively low frequency (2/24 or 8%); (2) most of the tumours with microsatellite alterations (5/7 or 71%) were of less common histopathological types (epithelial subtypes such as endometrioid and mixed serous and mucinous, or non-epithelial types such as malignant mixed Müllerian or germ cell tumours); (3) tumour-associated alterations were observed in 3/4 (75%) of the patients with stage I tumours *vs* 4/37 (11%) of the patients with stage II, III and IV tumours ($P = 0.01$); (4) tumour-associated microsatellite instability was found to occur with similar frequencies among patients with and without clinical features suggestive of familial disease, including positive family history, early onset, or multiple primary tumours. In summary, we have observed microsatellite alterations in the neoplastic tissues of ovarian cancer patients with diverse genetic backgrounds and clinicopathological features. The pattern of alterations is consistent with the possibility that multiple mechanisms may be responsible for microsatellite instability in ovarian neoplasms.

Keywords: microsatellite instability; ovarian neoplasms

Microsatellites are widely distributed repetitive DNA sequences composed of short, tandemly repeated nucleotide motifs. In some neoplasms, these sequences exhibit a form of genetic instability characterised by the gain or loss of repeat units at multiple independent loci. Such alterations have been observed to accumulate in cells defective for DNA repair activities (Parsons *et al.*, 1993; Umar *et al.*, 1994*a*) and occur with highest frequency in association with the familial cancer syndrome HNPCC (hereditary non-polyposis colorectal cancer) (Aaltonen *et al.*, 1993; Peltomäki, *et al.*, 1993; Risinger *et al.*, 1993). HNPCC families are characterised by a high frequency of colorectal and extracolonic malignancies of the gastrointestinal, upper urological and female genital tracts, often with early age of onset (Lynch *et al.*, 1993; Watson and Lynch, 1993). Human homologues of bacterial and yeast DNA mismatch repair genes have been located on chromosomes 2 (hMSH2, hPMS1), 3 (hMLH1) and 7 (hPMS2), and mutations have been identified at these loci in HNPCC patients (Fishel *et al.*, 1993; Leach *et al.*, 1993; Bronner *et al.*, 1994; Kolodner *et al.*, 1994, 1995; Liu *et al.*, 1994; Lynch *et al.*, 1994; Mary *et al.*, 1994; Nicolaides *et al.*, 1994; Papadopoulos *et al.*, 1994; Han *et al.*, 1995).

Microsatellite instability has also been observed in a variety of sporadic malignancies, including those of the colon, endometrium, stomach, pancreas, lung, bladder, kidney, oesophagus, and haematopoietic system (Gonzalez-Zulueta *et al.*, 1993; Han *et al.*, 1993; Ionov *et al.*, 1993; Risinger *et al.*, 1993; Thibodeau, *et al.*, 1993; Duggan *et al.*, 1994; Mao *et al.*, 1994; Meltzer *et al.*, 1994; Merlo *et al.*, 1994; Mironov *et al.*, 1994; Rhyu *et al.*, 1994; Shridhar *et al.*, 1994; Uchida *et al.*, 1994; Wada *et al.*, 1994). However, there are conflicting reports concerning the presence of such alterations in sporadic ovarian neoplasms (Dodson *et al.*, 1993; Han *et al.*, 1993; Osborne and Leech, 1994; Wooster *et al.*, 1994; King BL, Carter D and Kacinski BM. Microsatellite instability in tumours (unpublished data from The Fourth Meeting on the Molecular Basis of Cancer, Frederick, MD, USA, June 1993)). One analysis of more than 20 microsatellite markers failed to detect a single microsatellite alteration in any of 60 sporadic epithelial ovarian tumours (Dodson *et al.*, 1993). Another comprehensive allelotype analysis of 25 sporadic ovarian tumours at 68 different microsatellite loci revealed only two alterations among the 1700 repetitive sequences examined (Osborne and Leech, 1994). Together, these studies indicated microsatellite instability to be an extremely uncommon event in sporadic ovarian cancer. In contrast, another smaller study reported dinucleotide alterations in 3/19 (16%) ovarian tumours (Han *et al.*, 1993). Interestingly, another analysis failed to detect alterations at any of the six dinucleotide loci examined, but identified mutations at higher order tri- and tetranucleotide repeat sequences in 2 of the 20 ovarian tumours (Wooster *et al.*, 1994).

The interpretation of these conflicting observations is partially confounded by the variables of family history, tumour histopathology and type of microsatellite marker studied. Ovarian tumours occur with relatively high frequency in some HNPCC pedigrees (Lynch *et al.*, 1986), and tumour-associated microsatellite instability has been observed in an ovarian cancer patient with a germline hMSH2 mutation (Orth *et al.*, 1994). It is often difficult to obtain sufficient family history to exclude the possibility that cases assembled as sporadic are, in fact, from HNPCC pedigrees. Neither detailed histories nor clinicopathological characteristics were presented in two of the studies reporting tumour-associated microsatellite instability in sporadic ovarian tumours (Han *et al.*, 1993; Wooster *et al.*, 1994). The almost exclusive occurrence of alterations at higher order tri- and tetranucleotide repeat sequences in one of these studies suggested that features of the repeat loci themselves might be determinants of tumour-associated instability (Wooster *et al.*, 1994). In the present study, we have analysed di- and tetranucleotide microsatellite loci in DNA from the neoplastic and non-neoplastic tissues of 41 ovarian cancer patients characterised for family history and clinicopathological features.

Materials and methods

Patients and specimens

The clinical and histopathological characteristics of the 41 ovarian cancer patients analysed for microsatellite instability

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Received 23 November 1994; revised 24 March 1995; accepted 3 April 1995

Table I Clinicopathological characteristics of ovarian cancer patients

Patient no.	*Ovarian tumour histology*	*Micro satellite Instability*	*FIGO stage/ grade*	*Synchronous/ metachronous tumours*	*Age at onset*	*Family history*
1	Endometrioid adenocarcinoma	Yes	I,2	No	72	Breast/ endometrium
2	Mixed serous and mucinous adenocarcinoma	Yes	I,.5	Endometrium	64	Breast/ prostate
3	Malignant mixed Müllerian tumour	Yes	III,4	No	66	No
4	Endometrioid adenocarcinoma	Yes	I,1	No	36	No
5	Serous adenocarcinoma	Yes	III,3	Brenner tumour	79	No
6	Immature teratoma	Yes	III,3	Clear cell ovarian adenocarcinoma	48	No
7	Serous adenocarcinoma	Yes	III,1	No	22	No
8	Serous adenocarcinoma[a]	No[a]/Yes[b]	I,2	Endometrium[b]	56	No
9	Serous adenocarcinoma	No	III,3	No	26	No
10	Adenocarcinoma with mixed epithelial elements	No	III,3	No	64	No
11	Serous adenocarcinoma	No	III,3	No	39	Breast/ liver
12	Serous adenocarcinoma	No	III,3	Breast	78	No
13	Endometrioid adenocarcinoma	No	III,2	No	60	No
14	Serous adenocarcinoma	No	III,1	No	76	Breast
15	Endometrioid adenocarcinoma	No	II,1	Endometrium	54	Not known
16	Endometrioid adenocarcinoma	No	III,1	Lung, endometrium, breast	73	No
17	Serous adenocarcinoma	No	III,3	Endometrium	62	No
18	Malignant mixed Müllerian tumour	No	III,4	Endometrium	72	Not known
19	Serous adenocarcinoma	No	III,2/3	Endometrium	32	No
20	Serous adenocarcinoma	No	II,3	Endometrium	60	No
21	Serous adenocarcinoma	No	III,2	No	44	No
22	Dysgerminoma	No	IV,3	No	19	Breast
23	Mixed serous and mucinous adenocarcinoma	No	III,2	No	36	Ovary, colon
24	Serous adenocarcinoma	No	III,3	No	43	Colon
25	Serous adenocarcinoma	No	III,1	No	36	No
26	Serous adenocarcinoma	No	II,2	No	31	No
27	Endodermal sinus tumour	No	III	No	26	No
28	Serous adenocarcinoma	No	III,2	No	35	No
29	Serous adenocarcinoma	No	III,2	No	28	No
30	Serous adenocarcinoma	No	III,2	No	37	No
31	Serous adenocarcinoma	No	III,2	No	28	No
32	Serous adenocarcinoma	No	III,1	No	27	No
33	Serous adenocarcinoma	No	II,1	No	28	No
34	Serous adenocarcinoma	No	IV,3	Breast	57	Lung, brain
35	Serous adenocarcinoma	No	III,3	No	20	No
36	Serous adenocarcinoma	No	III,3	No	54	Ovary

Table I Continued

Patient no.	Ovarian tumour histology	Micro satellite Instability	FIGO stage/ grade	Synchronous/ metachronous tumours	Age at onset	Family history
37	Mucinous adenocarcinoma	No	III,2	No	38	Breast
38	Mixed serous and mucinous adenocarcinoma	No	IV,3	No	36	No
39	Serous adenocarcinoma	No	III, .5	No	19	No
40	Serous adenocarcinoma	No	III,3	No	37	Endometrium, pancreas
41	Mixed serous and mucinous adenocarcinoma	No	III,3	No	45	Endometrium

are presented in Table I. Nineteen cases were selected from the archives of the Department of Pathology at the Yale University Medical School, and 22 cases were obtained from the Frauenklinik der Albert-Ludwigs-Universität of Freiburg. Family history was determined by medical records. For the Freiburg cases, positive family histories were confirmed by histological diagnosis. Details on patients with positive family histories are presented in Table II. Formalin-fixed, paraffin-embedded archival specimens, including primary tumour, metastatic deposits, lymph nodes and normal tissues, were used for DNA extraction and microsatellite analysis. Microdissection was performed on some sections to separate neoplastic and non-neoplastic tissues. Serial H&E sections of all tissues were reviewed by pathologists (MLC, DC and JP).

Table II Microsatellite instability and family history

Ovarian patient no.	Tumour/relative(s)	Microsatellite instability
1	Breast–sister Endometrium–sister	Yes
2	Breast–sister Prostate–brother	Yes
11	Breast–maternal aunt, paternal grandmother Liver–father	No
14	Breast–ten female relatives	No
22	Breast–grandmother	No
23	Ovary–mother, grandmother Colon–father, grandmother	No
24	Colon–father, paternal grandmother, great grandmother	No
34	Lung–uncle Brain–brother	No
36	Ovary–mother, sister	No
37	Breast–mother, sister	No
40	Endometrium–aunt Pancreas–grandmother	No
41	Endometrium–mother, two aunts, two cousins, two nieces	No

DNA extraction

DNA extraction was performed according to Wright and Manos, (1990). Five-micron-thick paraffin tissues were scraped from histological slides, placed in Eppendorf tubes and deparaffinised through successive rinses in 400 µl volumes of xylene and absolute and 95% ethanols. Tissues were vortexed in each of these solutions for 15 s, and pelleted by microcentrifugation at top speed for 10 min. The final pellets were air dried overnight, resuspended in Manos buffer (50 mM Tris pH 8.5, 1 mM EDTA, 0.5% Tween 20) and incubated overnight at 37°C. Solutions were then heated to 95°C for 5 min, and next incubated with 200 µg ml^{-1} Proteinase K (Boehringer Mannheim, IN, USA) at 55°C for 3 h. Proteinase K was then heat inactivated in a 5 min 95°C incubation. Samples were stored at −20°C until use.

Microsatellites

The following microsatellites were amplified in radiolabelled polymerase chain reactions (PCRs) with the indicated primers: (1) the tetranucleotide $(GATA)_n$ GABARB1 locus on chromosome 4p12–13 (5′-tga tag cta gaa agc tag caa g-3′ and 5′-gct cat taa aca ctg tgt tcc t-3′) (Dean *et al.*, 1991); and (2) the dinucleotide $(CA)_n$ Mfd 27 locus on chromosome 5q11–13 (5′-gat cca ctt taa ccc aaa tac-3′ and 5′-ggc atc aac ttg aac agc at-3′) (Weber *et al.*, 1990).

PCR

PCRs were performed according to the specifications of the Perkin-Elmer Cetus Gene-Amp PCR reagent kit (Norwalk, CT, USA) with minor modifications. Briefly, 5 µl of the above DNA solutions was used for each 50 µl PCR reaction containing 1 × reaction buffer, 1.25 units of AmpliTaq DNA polymerase, 20 ng of each primer and 200 µM each of dCTP, dGTP and dTTP. The concentration of cold dATP per reaction was reduced to 50 µM, and 2.5 µCi [^{35}S]dATP (DuPont, NEN Products, Boston, MA, USA) was added. The reaction mixtures were cycled in a Perkin-Elmer Cetus DNA thermal cycler for 35 cycles consisting of a 1 min denaturing step at 94°C, a 1 min annealing step at 55°C and a 1 min extension step at 72°C. Ten microlitres of each completed PCR reaction were then mixed with 5 µl of sequencing stop solution (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol; United States Biochemical, Cleveland, OH, USA), and heat denatured at 80°C for 3 min. Three microlitre volumes of the denatured samples were resolved in 6% denaturing polyacrylamide sequencing gels (Sequagel, National Diagnostics, Manville, NJ, USA) subjected to 1500 V for 2.5 h. The gels were fixed in a 10% methanol–10% acetic acid solution for 1 h, heat dried in a vacuum gel drying apparatus and then autoradiographed using X-OMAT autoradiographic film (Kodak, Rochester, NY, USA) for 1–7 days.

Statistics

Statistical comparison of mutation frequencies associated with clinicopathological features was performed using Fisher's exact test.

Results

Microsatellite alterations

Microsatellite instability was observed in the malignant tissues of 7/41 (17%) of the ovarian cancer patients studied. In

all cases, alterations consisted of one or more additional novel alleles (Figure 1). More than one additional novel allele was observed in three of the tumours. Two of the seven novel alleles were larger than the wild-type allele, four were smaller and one was intermediate in length between the two wild-type alleles. In two of the cases, the novel allele differed by more than one repeat unit from the wild-type allele (Figure 2). Analysis of DNAs extracted from separate serial paraffin tissue sections was performed on six of the cases with alterations, confirming them in all cases.

All seven of the cases characterised for instability exhibited alterations at the tetranucleotide GABARB1 locus. Two of these cases (nos. 1 and 3) were found to exhibit instability at the dinucleotide Mfd 27 locus as well. One of the patients (no. 8) was diagnosed with synchronous tumours of the ovary and uterus. In this case, alterations were observed at both loci in the uterine tumour, but at neither in the ovarian tumour. In case no. 6, a novel allele was observed in the primary tumour, but not in a metastatic deposit (Figure 1). In case no. 2, a novel allele was observed in the DNA derived from one region of the ovarian tumour, but was absent in DNA derived from a remote region of the same tumour.

Familial disease

Family histories were obtained for 39 of the patients, twelve of whom were found to have relatives with cancer (Table II). Tumour-associated microsatellite instability was observed in 2/12 (17%) of these patients (nos. 1 and 2) *vs* 5/27 (18%) of the patients with negative histories ($P > 0.1$). Patient no. 1 had a positive family history for breast and endometrial cancer, and patient no. 2 had a positive family history for breast and prostate cancer. The remaining ten patients with positive family histories had relatives with a variety of malignancies, including ovarian, breast, endometrial and colon (Table II). One of these patients (no. 24) belonged to a pedigree fitting the classic definition of HNPCC, but was not observed to have tumour-associated microsatellite alterations. Twenty-five of the 41 patients (61%) were diagnosed with ovarian tumours before the age of 50. Tumours from three of these patients (12%) had microsatellite alterations, whereas tumours from 4/16 (25%) of the patients diagnosed after 50 were found to have such alterations ($P > 0.1$). Eleven patients were diagnosed with synchronous or metachronous tumours, and three of these (27%) were found to have microsatellite instability, whereas 4/30 (13%) without multiple primary neoplasms had tumour-associated microsatellite instability ($P > 0.1$).

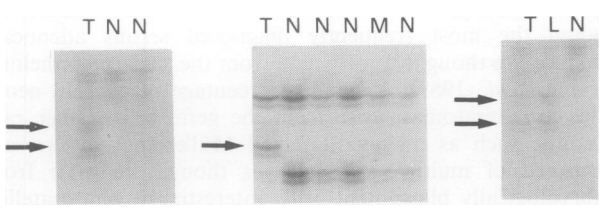

Figure 1 Autoradiograph demonstrating GABARB1 tetranucleotide microsatellite instability in three ovarian cancer patients: case no. 1 (left), case no. 6 (middle) and case no. 3 (right). DNAs were extracted from neoplastic and non-neoplastic tissues, amplified by radiolabelled PCR, and resolved in denaturing polyacrylamide gels. Arrows denote novel alleles. T, tumour; N, normal; M metastasis; L, lymph node.

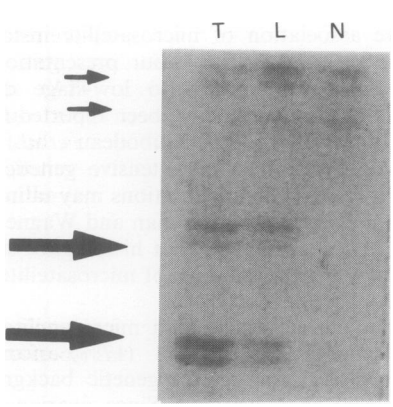

Figure 2 GABARB1 profiles for tumour (T), lymph node (L) and normal (N) tissues from ovarian cancer patient no. 4. The two wild-type alleles are denoted by small arrows, and two additional wild-type alleles are shown with large arrows. The novel alleles differ in length from the wild-type alleles by 8 and 16 bases. This pattern suggests that large loops may have been generated during microsatellite slippage events.

Pathology

The clinicopathological features of the tumours studied are presented in Table I. Twenty-four of the 36 epithelial ovarian neoplasms were classified as serous adenocarcinomas. Only two of these (2/24 or 8%) were found to have microsatellite alterations. The remaining five tumours in which microsatellite instability was observed were classified as endometrioid carcinomas (2), mixed serous and mucinous carcinomas (1), malignant mixed Müllerian tumour (1) and immature teratoma (1) (Table III). Five of the 36 (13%) epithelial *vs* two out of five (40%) non-epithelial tumours had microsatellite alterations. Thirty of the tumours studied were classified as FIGO stage III at presentation, of which four (13%) had alterations. None of the four stage II and none of the three stage IV tumours were found to have alterations. However, three of the four (75%) stage I presentations were characterised as positive for microsatellite instability ($P = 0.01$).

Normal tissues

No alterations were found in any of the non-neoplastic tissues analysed from this group of patients, with two exceptions. Two lymph nodes (from patients 3 and 4), characterised as histopathologically negative for metastatic involvement, were found to have microsatellite mutation patterns identical to those observed in the primary ovarian tumours (Figure 1). H&E staining failed to demonstrate the presence of epithelial elements, and immunohistochemical analysis failed to detect cells positive for cytokeratins A1 and A3 in either node. However, both of these lymph nodes were massively infiltrated by histiocytes. The presence of novel microsatellite alleles in the DNAs extracted from these nodes was interpreted as likely to have been derived from the residual DNA of phagocytosed ovarian carcinoma cells.

Discussion

We originally analysed microsatellites for the purpose of fingerprinting ovarian tumour cell lines (King *et al.*, 1994), and became curious about the general stability of these sequences *in vitro* and *in vivo*. We proceeded to study the

Table III Frequencies of microsatellite alterations according to histological classification

Epithelial	
Serous adenocarcinoma	2/24
Endometrioid adenocarcinoma	2/5
Mixed serous and mucinous carcinoma	1/4
Other	0/3
Non-epithelial	
Malignant mixed Müllerian	1/2
Immature teratoma	1/3

GABARB1 tetranucleotide locus in the tissues of a small group of ovarian cancer patients, and observed several alterations in the malignant tissues (King *et al.*, 1993, unpublished data). However, these observations appeared to contradict a larger analysis of 60 sporadic ovarian tumours which failed to detect alterations at any of the more than 20 repeat loci examined (Dodson *et al.*, 1993). Subsequent studies, investigating microsatellite instability in multiple tumour types, reported alterations in 3/20 (16%) and 2/20 (10%) of the ovarian tumours (Han *et al.*, 1993; Wooster *et al.*, 1994). The overall frequency of alterations observed in the present study (7/41 or 17%) is consistent with these two studies. Possible explanations of the divergent observations involve the variables of family history, microsatellite repeat features and tumour histology.

Although most ovarian cancer is thought to be sporadic, familial aggregation is recognised in three types of pedigrees (Lynch *et al.*, 1986; DiCioccio and Piver, 1992): (1) those with a high frequency of ovarian neoplasms alone; (2) those in which there is a high frequency of both ovarian and breast cancer; and (3) those characterised by a high frequency of adenocarcinomas of the colon, endometrium and ovary (e.g. HNPCC). In our study, family histories were available for 39 of the patients, and 12 of these patients had relatives with cancer (Table II). One patient had a family history meeting the Amsterdam criteria for HNPCC syndrome, i.e. three cases of colon cancer among closely related members of successive generations, with at least one case being diagnosed before the age of 50 (Vasen *et al.*, 1991). This patient was not found to have tumour-associated microsatellite instability. The other patients with positive family histories had relatives with a variety of malignancies, including ovarian, breast, endometrial and prostatic cancer (Table II). In all, tumour-associated microsatellite alterations were observed in only 2/12 (17%) of these patients. Similarly, the frequency of alterations was not significantly higher among patients with other clinical features suggestive of familial cancer, such as diagnosis before the age of 50 (12%) and the presence of synchronous or metachronous tumours (27%). Microsatellite instability did not, therefore, appear to be exclusively associated with features of familial ovarian cancer. Analyses of the hMSH2 and hMLH1 genes are currently being performed on the DNAs from cases showing tumour-associated microsatellite instability to determine if these patients have germline and/or somatically acquired mutations at these loci.

In theory, the frequency of detected microsatellite alterations depends on both the mutability of the repeat sequences under study and the proficiency of DNA replication and repair activities of the cells which contain them. HNPCC tumours have been characterised by the genome-wide alteration of dinucleotide repeat sequences, a form of instability attributed to mutations in a number of mismatch repair genes (hMSH2, hMLH1, hPMS1 and hPMS2) (Fishel *et al.*, 1993; Leach *et al.*, 1993; Bronner *et al.*, 1994; Nicolaides *et al.*, 1994; Papadopoulos *et al.*, 1994). In contrast, a different pattern of instability has been observed in a variety of non-HNPCC tumours, in which alterations were found with lower frequency, and almost exclusively at higher order tri- and tetranucleotide repeats (Wooster *et al.*, 1994). This pattern could result from two distinct phenomena. First, exceptionally high germline mutation rates have been observed for tetranucleotide repeat sequences (Mahtani and Willard, 1993; Weber and Wong, 1993). The exclusive detection of alterations at these loci may reflect a more subtle form of the repair deficiency that generates genome-wide dinucleotide instability in HNPCC tumours (Wooster *et al.*, 1994). Alternatively, recent *in vitro* observations suggest that small mismatches and large loops resulting from slippage events at repeat sequences may be recognised and repaired by different components of the DNA mismatch repair machinery (Umar *et al.*, 1994*b*). Small mismatches involving only a few bases would be more likely to arise in misaligned dinucleotide repeats, whereas large loops are more likely to result from slippage at tri- and tetranucleotide repeats. The novel tetranucleotide alleles shown in Figure 2 differ in length from the wild-type alleles by 8 and 16 bases respectively, and could have resulted from a defect in large loop repair. Although we have observed di- and tetranucleotide instability simultaneously in some tumours (Figure 3), a subset of malignancies, including some sporadic ovarian neoplasms, may be specifically defective for large loop repair activity.

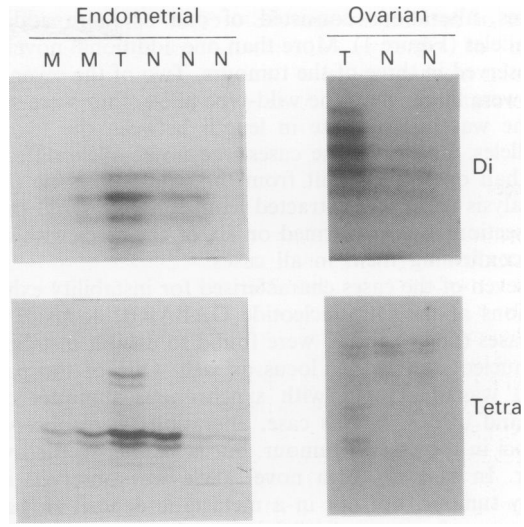

Figure 3 Simultaneous tetra- and dinucleotide microsatellite alterations in endometrial (case no. 8) and ovarian (case no. 1) tumours. T, tumour; N, normal; M, metastasis.

Ovarian neoplasms constitute a group of histopathologically diverse tumours. Most ovarian tumours, including the most frequently diagnosed serous adenocarcinomas, are thought to originate from the surface epithelium (Young *et al.*, 1989). A smaller percentage of ovarian neoplasias, e.g. teratomas, arise from the germ cells. Other rare tumours, such as malignant mixed Müllerian tumours, are composed of multiple cell lineages thought to arise from embryologically pluripotent cells. Interestingly, microsatellite instability was observed in more than half of these uncommon tumour types, and much less frequently in the more common serous adenocarcinomas (Table III). The two published studies reporting negligible frequencies of microsatellite alterations were done on epithelial ovarian carcinomas (Dodson *et al.*, 1993; Osborne and Leech, 1994), whereas histopathological classification was not provided in the studies reporting alterations (Han *et al.*, 1993; Wooster *et al.*, 1994). Histopathological classification may thus explain some of the discrepancies regarding microsatellite instability in ovarian cancer. Another interesting clinicopathological correlation was a positive association of microsatellite instability with the small number of stage I tumour presentations. Similar associations, linking alterations to low-stage disease and favourable patient prognosis, have been reported for colorectal tumours (Lothe *et al.*, 1993; Thibodeau *et al.*, 1993), and it has been suggested that the extensive genetic instability associated with microsatellite alterations may ultimately compromise tumour progression (Radman and Wagner, 1993). In short, our observations suggest that histological subtype and clinical stage may be determinants of microsatellite instability in ovarian neoplasms.

In conclusion, we have observed microsatellite instability in the neoplastic tissues of 7/41 (17%) ovarian cancer patients characterised for diverse genetic backgrounds and clinicopathological characteristics. Since ovarian cancer can be a manifestation of the HNPCC syndrome, it is possible that at least some of the patients in our study could be members of such pedigrees. This is particularly likely for patient no. 1, who had a positive family history of HNPCC-associated cancers and who was found to have alterations at di- and tetranucleotide repeat loci. However, tumour-associated microsatellite instability was also observed in a

number of patients without features of familial disease. The pattern of observed alterations suggests that multiple molecular mechanisms may be associated with the generation of microsatellite instability in ovarian neoplasms.

Acknowledgements
We thank Dr Franklin Hutchinson for statistical analysis and Ms Bettina Harris for clerical preparation of this manuscript.

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