

## Molecular and Clinicopathological Analysis of Ovarian Carcinomas With and Without Microsatellite Instability

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**Abstract.** *Background:* Microsatellite instability (MSI) occurs in sporadic ovarian carcinomas. This study tests the hypothesis that ovarian carcinomas arising through the mutator pathway have distinctive clinical and molecular features that affect clinical outcome. *Materials and Methods:* The MSI status was evaluated in 66 ovarian carcinomas and 11 epithelial ovarian tumors of low malignant potential. For the analysis with the microsatellite markers, a fluorescence-based PCR method was employed and the prognostic significance of the MSI status was assessed. DNA copy number changes in tumors with and without MSI were analyzed by comparative genomic hybridization. *Results:* High-frequency MSI (MSI-H) was found in 30% of the carcinomas, whereas low-frequency MSI (MSI-L) occurred in 32%. In LMP tumors, only MSI-L was detected (18%). There was a trend for tumors with MSI-H and MSI-L to have a poor prognosis, but this relationship did not reach significance ( $p=0.09$  and  $p=0.07$ , respectively). MSI-H in carcinomas was significantly associated with poor differentiation ( $p=0.03$ ) and higher clinical stage ( $p=0.03$ ). No correlation was found between different histological types of ovarian carcinoma and the microsatellite status. In a multivariate analysis, MSI at the dinucleotide repeat D5S346 was found to be of independent prognostic significance ( $p<0.008$ ) for disease-specific survival. There was no association between the total number of genetic aberrations per tumor and the MSI status. *Conclusion:* Microsatellite

instability is a relatively common event in ovarian carcinoma. The data indicate that instability of a single microsatellite marker on chromosome 5 (D5S346) might be indicative of disease progression when detected in early clinical stages.

Ovarian cancer is the deadliest of all gynecological malignancies in the Western world (1). The most important clinical prognostic factor is tumor stage, or extent of disease at diagnosis (2). Whereas women with organ-confined tumors have an excellent prognosis, most ovarian cancer patients show an advanced stage of the disease at the time of diagnosis and the overall 5-year survival for these women is less than 30%. Because ovarian cancer is frequently asymptomatic in its early stages, 75% of patients present with advanced-stage disease. Despite the development of new therapeutic approaches, the survival has remained largely unchanged for many years. If FIGO Stage I disease is a precursor of late-stage ovarian cancer, identifying molecular alterations in early-stage tumors should provide molecular markers for early detection (3).

Microsatellite instability (MSI) in cancers is characterized by inactivation of the DNA mismatch repair system, leading to a mutator phenotype in which simple repetitive DNA sequences, or microsatellites, are unstable during DNA replication (4, 5). In humans, at least six different mismatch repair genes have been described (hMSH2, hMSH3, hMLH1, hMSH6, hPMS2 and hPMS1). For mismatch recognition, the hMSH2 protein forms a heterodimer with two additional mismatch repair proteins, hMSH6 and hMSH3, depending on whether base-base mismatches or insertion/deletion loops are to be repaired (6-8). A heterodimer of hMLH1 and hPMS1 (or hMLH1 and hMLH3, or possibly hMLH1 and hPMS1) coordinates the interplay between the mismatch recognition complex and other proteins necessary for mismatch repair (9). The primary function of the post-replicative mismatch repair is to eliminate base-base mismatches and insertion/deletion loops that arise as a consequence of DNA polymerase

*Abbreviations:* MSI, microsatellite instability; MSI-H, high-frequency MSI; MSI-L, low-frequency MSI; CGH, comparative genomic hybridization; LOH, loss of heterozygosity; MMR, mismatch repair; LMP, low malignant potential.

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slippage during DNA synthesis (9-11). Cells with a defective mismatch repair system show a mutator phenotype in which many genes exhibit mutations because of uncorrected errors in DNA replication (12).

Mutations of mismatch repair genes (hMSH2, hMLH1, hMSH6, hPMS2, hPMS1) cause susceptibility to hereditary non-polyposis colorectal cancer (HNPCC). Predisposed individuals from HNPCC families have a high lifetime risk of developing colorectal carcinoma (85%), endometrial carcinoma (50%), ovarian cancer (10%), as well as other cancers, collectively referred to as the "HNPCC tumor spectrum" (13-15). Microsatellite instability also occurs in sporadic colorectal carcinomas. Sporadic as well as HNPCC-associated colorectal tumors displaying microsatellite instability show DNA diploidy, are at a low stage of development, show aggressive histological features, but have, paradoxically, a favorable outcome (9, 16).

Recently, high-frequency microsatellite instability was found to occur in 7-22% of sporadic ovarian carcinomas (17-23). To date, no association has been found between various histological types of ovarian carcinoma or the clinical outcome and the microsatellite status. Little data exists on the relationship between MSI and other molecular alterations in ovarian cancer (24). Therefore, we analyzed genomic alterations in ovarian carcinoma with and without MSI by comparative genomic hybridization (CGH). CGH allows the detection of DNA copy number changes across the genome and is applicable to all tumors regardless of their mitotic activity or the complexity of chromosomal changes (25). The method enables the detection of DNA sequence copy number changes if the affected region spans more than 10 Mb (26, 27). In addition, we evaluated the hMLH1, hMSH2 and hMSH6 expression in ovarian carcinomas with and without microsatellite instability.

## Materials and Methods

**Patients.** The study included 77 surgically resected ovarian tumors. The ovarian tumors were staged according to the fifth edition of the American Joint Committee on Cancer Staging System (28). Twenty-seven tumors were FIGO Stage I (35%), 14 tumors were FIGO Stage II (18%) and 36 tumors were FIGO Stage III (47%).

Histopathological analysis was performed by one author (H. M.) on hematoxylin and eosin-stained sections. Tumors were graded using a modified Broder's classification with invasive grades 1 and 2 considered low-grade (LG), and invasive grades 3 considered high-grade (HG), as previously applied and proposed in ovarian carcinoma (29, 30). Eleven tumors were tumors of low malignant potential (LMP). The remaining 66 tumors were malignant: 24 serous papillary carcinomas (9 Grade I/II, 15 Grade III; 1 Stage I, 7 Stage II, 16 Stage III), 21 endometrioid carcinomas (11 Grade I/II, 10 Grade III; 8 Stage I, 2 Stage II, 11 Stage III), 11 mucinous carcinomas (10 Grade I/II, 1 Grade III; 6 Stage I, 5 Stage III), 7 clear cell carcinomas (6 Grade I/II, 1 Grade III; 4 Stage I, 1 Stage II, 2 Stage III) and 3 other carcinomas (1 Grade I/II, 2 Grade III; 1 Stage II, 2 Stage III).

Follow-up information, information about metastasis and causes of death were obtained from the Department of Gynecology, University of Basel, and from the Basel Cancer Registry, Switzerland. Survival time was available for 49 out of 66 women with ovarian carcinomas and for all patients with ovarian tumors of low malignant potential. Survival time was calculated from the time of diagnosis to the death of patients.

**Tissue preparation.** Tissue samples of 77 ovarian carcinomas were fixed in 4% buffered formalin, embedded in paraffin and routinely stained for histological diagnosis. Specimens were trimmed to enrich for tumor cells by excising tumor tissue from the paraffin block. The excised tumor tissue was re-embedded in a paraffin block. Five- $\mu$ m tissue sections were cut from these tumor blocks. The first and the last sections were stained with hematoxylin and eosin to verify the presence of at least 75% tumor cells in the sample.

**Microsatellite analysis.** Genomic DNA was isolated using the Dneasy Tissue kit (Qiagen AG, Basel, Switzerland). Tissue probes from normal organs (colon, uterus) were obtained during surgery and were used as a source of normal DNA. For microsatellite analysis, five microsatellites (BAT25, BAT26, D2S123, D5S346, D17S250) had been proposed by the National Cancer Institute (NCI) as standard primer for investigations in colorectal cancer (31, 32). In this study, microsatellite instability in ovarian tumors was assessed using this panel of five microsatellite markers (17) recommended by the NCI. Two additional microsatellite markers (BAT40, MYCL1) were applied in MSI-L or MSS tumors. In addition to the NIH consensus marker panel, BAT40 was selected because this marker belongs to the markers which are most informative, whereas MYCL1 belongs to the marker panel of the second choice. PCR products were analyzed using a LI-COR DNA Sequencer (Model 4200, LI-COR Inc., Lincoln, Nebraska, USA).

MSI was defined as any change in length in a microsatellite amplified from a tumor when compared to normal tissue. Tumors were classified as tumors with high-frequency MSI (MSI-H) if two or more of the five NCI markers showed instability or if 30% or more of all markers tested demonstrated instability. Tumors were characterized as low-frequency MSI (MSI-L) tumors if only one of the five NCI markers showed instability or <30% of all of the markers. All of the other tumors were considered microsatellite stable (MSS) in at least two analyses.

**CGH analysis.** CGH was performed as described (25, 33, 34). At least four observations per autosome and at least two observations per sex chromosome were included in each analysis according to previous recommendations by Kallioniemi *et al.* (27). CGH experiments included a tumor cell line (Spectrum-Green labeled MPE-600 DNA; VYSIS) with known aberrations (positive control) and a hybridization of two differentially-labeled sex-mismatched normal DNAs to each other (negative control). The thresholds used for definition of DNA sequence copy number gains and losses were based on the results of CGH analyses of formalin-fixed normal tissues. Gains of DNA sequences were defined as chromosomal regions where both the mean green to red fluorescence ratio and its standard deviation (SD) were above 1.20, whereas losses were defined as regions where both the mean and its SD were below 0.80. Overrepresentations were considered amplifications when the fluorescence ratio values in a sub-region of a chromosome arm exceeded 1.5.

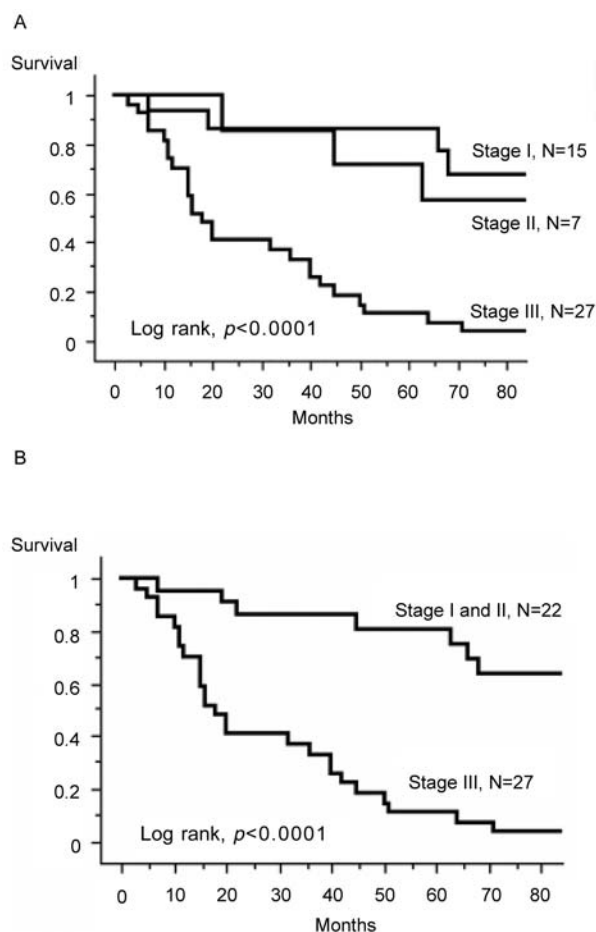


Figure 1. Overall survival in 49 patients with ovarian carcinoma. A. FIGO Stages I-III. B. FIGO Stages I and II versus Stage III.

**Immunohistochemistry.** To analyze expression of the repair proteins hMLH1, hMSH2 and hMSH6, an immunohistochemical analysis was performed using the ABC-Method (ABC-Elitekit, Vector, Burlingame, CA, USA). The sections were incubated with the primary antibodies to hMLH1 (PharMingen/Becton Dickinson, Basel, Switzerland, 1:100), to hMSH2 (Oncogene/Calbiochem, Schwalbach, Germany, 1:50) and to hMSH6 (Transduction Laboratories/Becton Dickinson, Basel, Switzerland, 1:500). The immunologic reactions were visualized with 3,3'-Diamino-benzidine (DAB) tetrahydrochloride (Serva-Chemie, Brunswick, Basel, Switzerland). Sections were counterstained with hematoxylin, dehydrated and, finally, mounted with Crystall/Mount (BiØmeda, Foster City, CA, USA). Normal stromal cells showing a clear nuclear staining were used as a positive control. Nuclear staining was considered as positive.

**Statistics.** Results are given as mean values and standard deviation (SD). Relationships between categorical features and counts were evaluated by the non-parametric method of the Mann-Whitney *U*-test. Multiple comparisons were performed by Fisher's test. Contingency table analysis was used to analyze the correlations among ovarian carcinomas with and without microsatellite instability,

tumors with and without mismatch repair protein expression, histological grade and FIGO stage. Survival was defined as the time between primary treatment and death. Patients that survived were censored at the time of their last follow-up. Survival analysis was completed using the Kaplan-Meier method with a log rank test. The median values of the numbers of DNA aberrations, DNA sequence losses and gains were used as cut-off points to define patients with high and low numbers of corresponding aberrations. A Cox proportional hazards analysis was used to test for independent prognostic information. Statistical analyses were performed by use of the StatView 5 Software program (Abacus Concepts).

## Results

**Tumors.** The set of 77 ovarian tumors was grouped into 11 ovarian tumors of low malignant potential (LMP) and 66 ovarian carcinomas. Complete cytoreduction during surgical treatment was accomplished in 83% of patients. The ovarian carcinomas were stratified by clinical stage (31 FIGO Stage I and II tumors; 35 FIGO Stage III tumors) and histological grade (36 LG tumors; 30 HG tumors). Survival analysis revealed that only very small differences were observed between Stage I and Stage II tumors (Figure 1A), as well as between MSI-H and MSI-L tumors (Figure 2). For further analysis Stage I and II tumors were grouped together to Stage I/II tumors (Figure 1B).

In ovarian carcinoma with follow-up information, 13 out of 17 tumors without progression following the primary treatment were LG tumors, whereas 18 out of 32 patients with unfavorable outcome had HG tumors.

All LMP tumors were grade I tumors and of FIGO Stage I. No disease progression was observed in women with LMP tumors, with a mean follow-up time of  $8.8 \pm 4.8$  years. Therefore, survival analysis to study the association of mismatch repair protein expression and the microsatellite status with the clinical outcome was restricted to patients with ovarian carcinomas, with a mean follow-up time of  $4.3 \pm 3.8$  years. Women who died due to cancer progression had a mean follow-up of  $2.4 \pm 1.7$  years.

**MSI status in carcinomas and LMP tumors.** According to the definitions of MSI, 20 out of 66 ovarian carcinomas (30%) showed instability at two or more microsatellite loci and were classified as MSI-H carcinomas. Twenty-one carcinomas (32%) were characterized by low-frequency microsatellite instability (MSI-L) with instability at only one locus.

Microsatellite instability was more frequent in ovarian carcinomas than in LMP tumors ( $p < 0.006$ ). No MSI-H occurred in tumors of low malignant potential. Only 2 out of 11 (18%) LMP tumors were classified as MSI-L tumors. For further assessment, all LMP tumors were excluded from survival analysis, since no tumor-related death occurred in women with LMP tumors.

Ovarian carcinomas with MSI-H were more likely to be poorly-differentiated ( $p = 0.03$ ) and to have a significantly higher

Table I. Clinical stage, histological classification and microsatellite status in epithelial ovarian tumors. The p-values of the statistical analysis of all epithelial ovarian tumors (including ovarian tumors with LMP) are shown in brackets.

Characteristic	N	MSI %	MSI-H	MSI-L	MSS	Chi square
FIGO Stage						$p < 0.09$
Stage I	27 (35.1%)	37%	3 (11%)	7 (26%)	17 (63%)	( $p < 0.02$ )
Stage II	14 (18.2%)	57%	5 (36%)	3 (21%)	6 (43%)	
Stage III	36 (46.7%)	69%	12 (33%)	13 (36%)	11 (31%)	
Grade						$p < 0.09$
I/II (LG)	48 (62%)	44%	7 (15%)	14 (29%)	27 (56%)	( $p < 0.01$ )
III (HG)	29 (38%)	76%	13 (45%)	9 (31%)	7 (24%)	
Histology						$p < 0.2$
Serous papillary	24 (31.2%)	71%	11 (46%)	6 (25%)	7 (29%)	( $p < 0.03$ )
Endometrioid	21 (27.3%)	52%	3 (14%)	8 (38%)	10 (48%)	
Mucinous	11 (14.3%)		3	3	5	
Clear cell	7 (9.1%)		1	3	3	
Tumors of LMP	11 (14.3%)	18%	0	2	9 (82%)	
Other	3 (3.4%)		2	1	0	

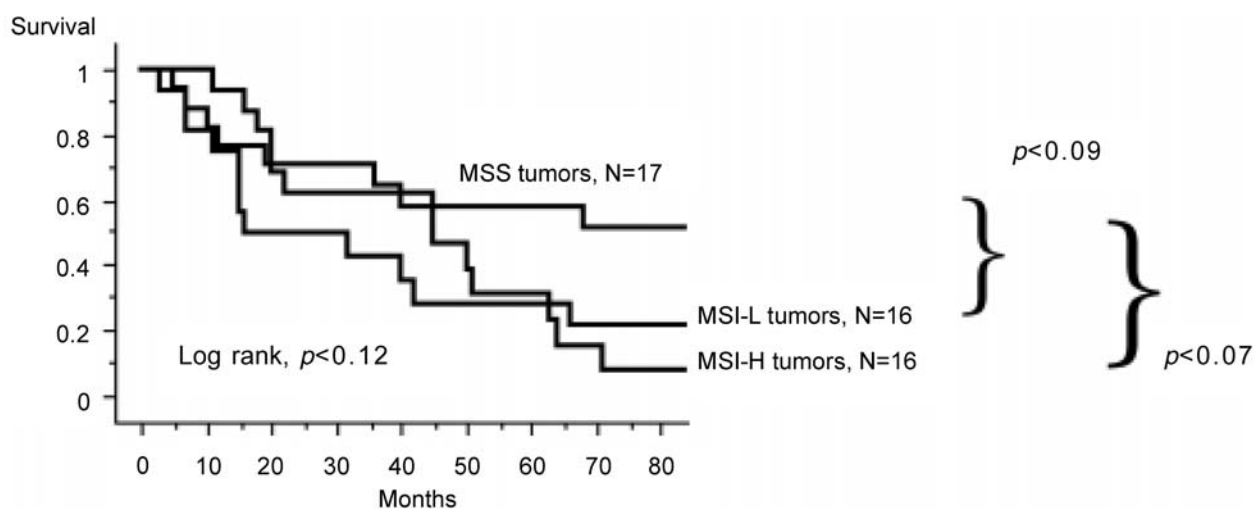


Figure 2. Overall survival in patients with ovarian carcinoma stratified according to the microsatellite status. MSS (stable), MSI-L (low-frequency MSI) and MSI-H (high-frequency MSI) tumors.

overall clinical stage ( $p=0.03$ ). The MSI status and the presence of residual carcinoma following the surgical exploration were significantly related to the FIGO Stage. Residual cancer tissue, *i.e.* cancer tissue spread beyond the ovaries, was more likely in MSI carcinomas than in MSS carcinomas ( $p < 0.004$ ). No correlation was found between different histological types of ovarian carcinoma and the microsatellite status (Table I).

**MSI status and clinical outcome.** Thirty-two out of the 49 patients (65%) died during the follow-up period. The survival of patients with microsatellite instability was worse than that of patients with MSS tumors (Figure 2). Both MSI-L and MSI-H

tumors were associated with poor survival, but this relationship did not reach significance ( $p < 0.09$  and  $p < 0.07$ , respectively).

All microsatellite markers were individually tested for a relationship with tumor-related survival. Instability of the dinucleotide repeat D5S346 was significantly associated with survival in both the FIGO Stages I/II (Figure 3A and 3B) and in FIGO Stage III (Figure 3C). When individually tested, none of the other markers were related to survival.

In a stepwise multivariate analysis, clinical stage, instability of the microsatellite marker D5S346, level of cyto-reduction and overall status of MSI were found to be significantly and independently associated with survival (Table II).



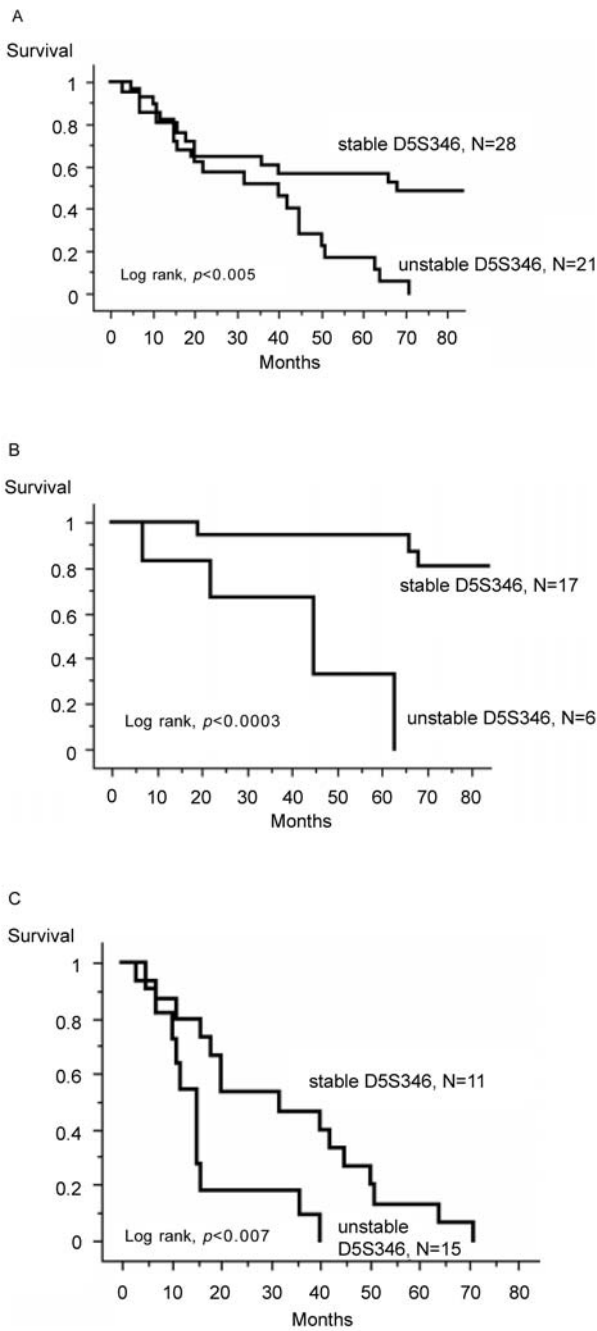


Figure 3. Overall survival in patients with ovarian carcinoma stratified according to changes in the dinucleotide repeat D5S346. A. D5S346 status in all cancers irrespective of their FIGO stage. B. D5S346 status in the combined Stage I/II carcinomas. C. D5S346 status in carcinomas with Stage III.

**CGH analysis.** Genomic aberrations were detected by CGH in 19 epithelial carcinomas. In this set of tumors no significant differences were observed in the number of DNA aberrations between tumors of different clinical stages, nor between

Table II. Cox proportional hazard analysis for ovarian carcinomas.

Variable	Risk ratio	p-value
FIGO Stage	21.43	<0.0001
D5S346	7.07	<0.008
Level of cytoreduction	5.36	0.02
MSI Status	3.96	0.04
Grade of differentiation	2.31	0.13

Table III. CGH analysis in ovarian carcinomas. Comparison of DNA copy number changes with the microsatellite status, clinical stage and histological grade (Mann-Whitney test).

	N	All copy number changes/tumor	p	DNA losses per tumor	p	DNA gains per tumor	p
FIGO Stage			0.3		0.4		0.6
Stage I and II	9	5.6±3.8		4.3±2.7		1.4±1.5	
Stage III	10	4.2±4.8		3.2±3.6		1±1.6	
Histolog. Grade			0.9		0.7		0.5
Grade I/II (LG)	8	4.7±3.7		3.8±2.7		0.9±1.3	
Grade III (HG)	11	5.2±4.9		3.7±3.5		1.4±1.8	
MSI Status			0.5		0.6		0.4
MSS tumors	6	3.9±2.7		3.1±2.2		0.8±1.2	
MSI tumors	13	5.6±5		4.1±3.6		1.5±1.7	

tumors of different histological grades. There were no significant differences in the overall frequency of DNA copy number changes between MSI and MSS tumors (Table III).

However, there were differences in DNA copy number changes on individual chromosomal locations. In MSI carcinomas, DNA losses (>20%) occurred most frequently on chromosome 2p (44.4%), 8p (33.3%), 17p (33.3%), 6p, 10q, 14q, 15q and 16p (22.2% each). DNA gains were observed on 4q (33.3%). MSS carcinomas showed DNA losses mostly on 3p (33.3%), Xp (33.3%) and 5q (25%), whereas DNA gains were found on 12q (25%). There was a trend for DNA losses on chromosomes 2p, 3p, 4q and 17p to be more frequent in MSI carcinomas. However, the number of tumors was too small to determine significant differences.

**Mismatch repair protein expression in MSI and MSS ovarian carcinomas.** Nuclear immunoreactivity for hMLH1, hMSH2 or hMSH6 proteins was observed in all 25 MSS carcinomas. Twenty-seven out of 41 (65%) MSI carcinomas displayed expression of hMLH1, hMSH2 and hMSH6. Complete loss of hMLH1, or hMSH2, or hMSH6 expression was detected in 10 out of 41 (24%) carcinomas with MSI (Table IV). No differences were observed in the mismatch repair protein expression between different clinical stages or different histological grades. The hMLH1, hMSH2 and MSH6 expression was not related to survival.

Table IV. Distribution of MMR protein expression and microsatellite marker instability in 66 women with invasive ovarian carcinoma. Percentages are given for the total number of cases in each subgroup.

	MSI-H (n=20)	MSI-L (n=21)	p-value Chi- square	MSI H+L (n=41)	MSS (n=25)	p-value Chi- square
<b>MMR proteins</b>						
hMLH1	14 (70%)	17 (81%)	0.6	31 (76%)	24 (96%)	0.03
hMSH2	17 (85%)	14 (66%)	0.07	31 (76%)	20 (80%)	0.7
hMSH6	16 (80%)	15 (71%)	0.3	31 (76%)	22 (88%)	0.2
<b>Microsatellite marker</b>						
BAT25	5 (25%)	0		5 (12%)	0	
BAT26	3 (15%)	0		3 (7%)	0	
D5S346	17 (85%)	8 (38%)	0.002	25 (61%)	0	
D2S123	12 (60%)	2 (9%)	0.0007	14 (34%)	0	
D17S250	13 (65%)	11 (52%)	0.4	24 (58%)	0	
BAT40	1 (5%)	0		1 (2%)	0	
MYCL1	0	0	-	0	0	-

## Discussion

This study demonstrates that the phenomenon of MSI in ovarian carcinomas differs from MSI in colorectal carcinomas. Sporadic colorectal carcinomas with MSI are characterized by a specific phenotype, better prognosis, reduced rate of DNA aneuploidy and a lower number of DNA aberrations per tumor compared to their MSS counterparts (35, 36). Our data suggests that the subgroup of ovarian carcinomas with MSI is characterized by poor prognosis. The results of the survival analysis might indicate that low-frequency MSI share a similar biological relevance to high-frequency MSI ovarian carcinomas. Tumors with this genetic phenotype were more likely to have an advanced pathological stage and a poor differentiation grade.

This is in contrast to reports for both heritable and sporadic types of colorectal cancer where MSI is related to improved survival (37, 38). The exact mechanism by which MSI influences clinical outcome is unknown, but it may be related to the kinds of mutations or the genetic targets involved in ovarian carcinogenesis that are deficient in DNA mismatch repair. Thus, microsatellites in cancer cells undergo expansion or contraction at high frequencies. Contraction or expansion of repeats within genes provide a mechanism for inactivation of tumor suppressor genes during tumor progression (4).

In this study, the dinucleotide repeat D5S346 (5q22-q23), located in close proximity to the APC gene, was unstable in 38% of all ovarian carcinomas. Interestingly, the microsatellite marker D5S346 was an independent predictor of clinical outcome. It may prove valuable to possess markers that identify the subgroup of ovarian carcinomas

Table V. Frequencies of microsatellite alterations obtained from selected previous analyses of the microsatellite status in unselected sporadic ovarian carcinomas.

Author	N	MSI-H N	MSI-H %	Histology	Survival
Gras, 2001 (20)	56	7	13	No correlation	-
Watanabe, 2001 (51)	24	2	8	-	-
Alvi, 2001 (21)	43	0	0	-	-
Allen, 2000 (19)	26	1	4	-	-
Hickey, 1999 (52)	20	2	10	-	-
Tangir, 1996 (53)	31	2	6	-	-
Sood, 2001 (17)	109	13	12	-	No correlation
Buller, 2001 (18)	100	22	22	-	-
Own study*, 2003	77	20	26		

\* including tumors of LMP

(-) no correlation provided in study

with microsatellite instability. Markers with high sensitivity and specificity for the identification of MSI carcinomas would reduce the number of markers needed to identify this phenotype and increase the feasibility of such testing. Although larger studies should be conducted, the findings reported here suggest that D5S346 instability might be a specific single marker for the MSI phenotype in ovarian carcinomas.

There are conflicting reports on the frequency of microsatellite instability in ovarian carcinomas. While a range of authors report an overall frequency of high-frequency MSI of between 10-20% (17, 23, 39, 40), a low prevalence of MSI (19-21, 41, 42) or almost no involvement of size changes in nucleotide repeats have been reported in ovarian carcinomas. We found a relatively high overall percentage of MSI in carcinomas. In our study, MSI-H was observed in 30% of ovarian carcinomas. This rate was higher than the rate published in previous studies (Table V). In contrast to these recently published studies, we used 7 microsatellite markers and not only the 5 of the recommended marker panel. This might explain the higher prevalence of MSI in our tumor set. Our results support the distinction between MSI-L and MSS carcinomas, as well as between MSI-L and MSI-H carcinomas (43). The clearly different patterns of instability in mono- and dinucleotide markers between MSI-L and MSI-H tumors, as well as the independent significance of D5S346, justify their separation.

To date, few cytogenetic investigations have been published that address the question of differences in chromosomal changes of ovarian carcinomas with and without microsatellite instability (22, 44, 45). In this

study, there was no association between the number of genomic aberrations and the status of microsatellite instability in ovarian carcinomas. This is in contrast to previously published molecular genetic data in colorectal carcinomas. Schlegel *et al.* (36) found statistically significantly more gross chromosomal aberrations by CGH in MSS than in MSI colorectal carcinomas. MSI colorectal tumors rarely demonstrated numeric genetic alterations. None of them showed gene amplifications and DNA losses were found in only 2 out of 6 MSI tumors. In contrast, the majority of MSI-negative tumors exhibited gains and recurrent deletions. In our study, different individual genomic aberrations between MSI and MSS ovarian carcinomas were found, but the number of tumors was too small to define significant differences in the involved chromosomal regions.

This study investigated the expression of three mismatch repair proteins by immunohistochemistry. While hMLH1 functions as a nuclear-encoded protein in mitochondrial mismatch repair, both hMSH2 and hMSH6 function in nuclear mismatch repair. Germ line mutations in hMSH2 and hMLH1 account for the majority of HNPCC families, implicating mismatch repair in the etiology of the inherited disease. Interestingly, mutations of hMSH6 appear to be rare (46) and to be associated with endometrial carcinomas (47). In humans, hMSH2 and hMSH6 form a heterodimer which specifically binds single-mispaired nucleotides and a subset of nucleotide insertion-deletion mismatches. It has been suggested that most, if not all, of hMSH6 is bound in complexes with hMSH2 (8, 46). Mutations in hMLH1 or in the hMSH2-hMSH6 heterodimeric complex may account for the loss of immunoreactivity in a small subset of MSI ovarian carcinomas. Recently reported data suggested that hMSH2 might be involved in the onset or progression in a subset of ovarian cancer (48). Geisler *et al.* found that hMSH2 is an independent predictor of survival in 102 patients with ovarian carcinoma (49). In colorectal carcinomas, a close relationship between MMR protein expression by immunohistochemistry and MSI status was reported (32). Our data demonstrates that loss of MMR gene products occurs in MSI ovarian carcinomas, whereas MSS carcinomas usually express all MMR proteins. Thus, loss of the MMR protein might indicate microsatellite instability in a small subset of sporadic ovarian carcinomas. This would be consistent with recently reported data (50).

In conclusion, our data indicate that microsatellite instability is a relatively common event in ovarian carcinoma and that MSI might be associated with unfavorable outcome in ovarian carcinomas. Instability of a single microsatellite marker on chromosome 5 (D5S346) appears to be indicative of disease progression when detected in early clinical stages.

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