Microsatellite Instability Analysis: A Multicenter Study for Reliability and Quality Control

Tina Bocker, Johannes Diermann, Waltraut Friedl, et al.

Cancer Res 1997;57:4739-4743.

[CANCER RESEARCH 57, 4739–4743, November 1, 1997]

Advances in Brief

Microsatellite Instability Analysis: A Multicenter Study for Reliability and Quality Control[1]

Tina Bocker,[2] Johannes Diermann, Waltraut Friedl, Johannes Gebert, Elke Holinski-Feder, Judith Karner-Hanusch, Magnus von Knebel-Doeberitz, Konrad Koelble, Gabriela Moeslein, Hans-Konrad Schackert, Hans-Christian Wirtz, Richard Fishel,[2] and Josef Rüschoff[2]

Kimmel Cancer Institute, Thomas Jefferson University, Philadelphia, Pennsylvania 19107 [T. B., R. F.]; Chirurgische Klinik I, D-12101, Berlin Tempelhof, Berlin, Germany [J. D.]; Institut fuer Humangenetik, Universitaet Bonn, D-53111, Bonn, Germany [W. F.]; Chirurgische Klinik der Ruprecht-Karls-Universitaet Heidelberg, D-69123, Heidelberg, Germany [J. G., M. v. K-D.]; Abteilung fuer paediatrische Genetik der Kinderpoliklinik der LMU Muenchen, D-80336, Munich, Germany [E. H-F.]; Chirurgische Universitaetsklinik Wien, A-01090, Vienna, Austria [J. K-H.]; Institut fuer Pathologie der Charité and Max-Delbrück-Centrum fuer molekulare Medizin, D-10117, Berlin, Germany [K. K.]; Chirurgische Klinik [G. M.] and Institut fuer Pathologie Heinrich-Heine-Universitaet, D-40225, Dusseldorf, Duesseldorf, Germany [H-C. W.]; Chirurgische Forschung TU Dresden, D-01307, Dresden, Germany [H-K. S.]; and Institut fuer Pathologie der Universitaet Regensburg, D-93042 Regensburg, Germany [J. R.]

Abstract

The molecular biology section of the Hereditary Non-Polyposis Colorectal Cancer study group–Germany, instituted a multicenter study to test the reliability and quality of microsatellite instability (MSI) analysis. Eight laboratories compared MSI analyses performed on 10 matched pairs of normal and tumor DNA from patients with colorectal carcinomas. A variety of techniques were applied to the detection of microsatellite changes: (*a*) silver and ethidium bromide staining of polyacrylamide gels; (*b*) radioactive labeling; and (*c*) automated fluorescence detection. The identification of highly unstable tumors and tumors without MSI was achieved in high concordance. However, the interpretation of the band patterns resulted in divergent classifications at several microsatellite marker loci for a large fraction of this tumor/normal panel.

The data on more than 30 primers per case suggest that the enlargement of the microsatellite panel to more than 10 loci does not influence the results. In this study, cases with MSI in less than 10% of loci were classified as microsatellite stable, whereas MSI was diagnosed in cases with more than 40% of all markers unstable. We propose that a panel of five microsatellite loci consisting of repeats with different lengths should be analyzed in an initial analysis. When less than two marker loci display shifts in the microsatellite bands from tumor DNA, the panel should be enlarged to include an additional set of five marker loci. The number of marker loci analyzed as well as the number of unstable marker loci found should always be identified. These criteria should result in reports of MSI that are more comparable between studies.

Introduction

Microsatellites are defined as short tandemly repeated sequences that occur randomly throughout the genome. Many of these microsatellite sequences are highly polymorphic between individuals (1). The length of the repeated motifs ranges from 1 to 6 bases, which can be iterated several to hundreds of times (1). However, their evolution and function in the genome is not quite clear. As a result of their ubiquitous occurrence on all chromosomes, microsatellites have been used by human geneticists as a method of mapping the genome and to study the segregation of alleles with respect to suspected inherited diseases. During the last 4 years, however, somatic MSI[3] has also been detected in a variety of tumors (2). Alterations in the length of the microsatellite alleles in tumor DNA compared to constitutive DNA from the same patient can be detected by PCR and visualized with different techniques (3, 4).

MSI is especially evident in tumors from patients with HNPCC, where it is found in approximately 90% of cases (5). In addition, sporadic colorectal carcinomas display this phenomenon in approximately 15–30% of cases (6, 7). However, it is the absence of MSI that is a valuable predictor for the absence of HNPCC because alteration of the human mismatch repair genes has been found to be responsible for the MSI of these HNPCC tumors (8). It has been proposed that it is the loss of mismatch repair function that results in an inability to recognize slip-mispaired intermediates that ultimately result in MSI (9, 10). At present, the diagnosis of the HNPCC syndrome implies the sequencing of up to four mismatch repair genes, which is a time- and cost-consuming procedure. Microsatellite analysis can be used as an initial screening method before the mismatch repair genes themselves are analyzed for germ-line mutations. It must be emphasized that the finding of MSI is not sufficient for the diagnosis of HNPCC or hereditary cancer (8).

No consensus exists in how many loci should be analyzed and how many of them should show alterations to be classified as MSI. A variety of methods for microsatellite analysis has been established, and thousands of primer sequences have been published, which can be used for the amplification of mono-, di-, tri-, tetra- and other oligonucleotide repeats.

The molecular biology section in the HNPCC study group–Germany has attempted to compare different methods for microsatellite analyses. The goal of these studies was: (*a*) to compare the reliability of MSI with respect to the different methodology and interpretation, performed in different laboratories; (*b*) to develop parameters for quality control and to determine whether cases with microsatellite instability were identified equivalently; and (*c*) to deduce the minimal number of loci that must be studied to classify a tumor as highly unstable or stable.

Materials and Methods

Two sets of five DNAs from colorectal tumors and corresponding normal colon mucosa were isolated at the Regensburg laboratory from frozen tissue blocks as described previously (11). Cases with and without MSI, including cases with instability at single loci, were selected from a prospective series of

Received 8/5/97; accepted 9/22/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Presented by the HNPCC Study Group–Germany.

[2] To whom requests for reprints should be addressed: T. B.; R. F.: Kimmel Cancer Institute, Thomas Jefferson University, 233 South 10th Street, Philadelphia, PA 19107. E-mail: T_Bocker@lac.jci.tju.edu, rfishel@hendrix.jci.tju.edu. J.R. Institut fuer Pathologie, Universitaet Regensburg, Franz-Josef-Strauss-Allee, D-93042 Regensburg, Germany. E-mail: josef.rueschoff@klinik.uni-regensburg.de.

[3] The abbreviations used are: MSI, microsatellite instability; HNPCC, hereditary nonpolyposis colorectal carcinoma; MSS, microsatellite stable; LOH, loss of heterozygosity.

colorectal carcinomas (12). In this study, microdissection was not attempted because a large amount of DNA was required for distribution to all institutions. The only information given to the institutions was a case number and the information "tumor" (T) or "normal" (N), respectively. Independently and retrospectively to the multicenter study, the tissue expression of hMSH2 and hMLH1 was assessed by immunohistochemistry in all of the 10 cases as described elsewhere (13). All MSS and "low MSI" cases were found to express both hMSH2 and hMLH1 in normal and tumor tissue. Of the three cases with MSI, patients 6 and 7 had lost expression of hMSH2, and patient 4 had lost expression of hMLH1 in the tumor. Identification of the genetic alteration that led to loss of expression of these proteins in these respective tumors is under study.

In the first part of the study, each participant was asked to use the primer panel and the detection method routinely applied in his laboratory to analyze five paired colorectal tumor/normal DNA samples. Taken together, the eight participating laboratories used 33 different microsatellite primer pairs. The number of individual microsatellite loci analyzed by each participating group varied from 3 to 8 (Table 1A). The microsatellite locus at *D5S346* was analyzed by five groups, whereas *D2S123* and *D18S34* were each studied by four groups, and *D5S82* was examined by three groups. The PCR products were visualized on polyacrylamide gels by either silver or ethidium bromide staining (two groups each) or as a radioactive PCR product using ^{32}P-labeled oligonucleotide primers (two groups). Two additional groups used an automated DNA sequencer (Applied Biosystems; ALF, Pharmacia). The results were compared at a meeting where the clinical data were presented as well.

In the second part of the study, the participants were asked to analyze the microsatellite loci *D2S123* and *D5S346* and a mononucleotide repeat, preferably *BAT25,* in addition to a microsatellite panel of their choice on a second set of five paired colorectal tumor/normal DNA samples. In this part of the study, a maximum of 31 loci was studied (Table 1B). Again, different methods of visualization were applied: silver staining, ethidium bromide staining, and ^{32}P-labeling (one group each), and automated fluorescent microsatellite analysis on a DNA sequencer (three groups on either ALF or ABI). In this part of the study, *D2S123* was analyzed in six laboratories, *D5S346* in five laboratories, and *BAT25, D18S34,* and *BAT26* by three groups each. The data were compared and analyzed at the subsequent meeting of the HNPCC group.

MSI was defined by the presence of novel bands following PCR amplification of tumor DNA that were not present in PCR products of the corresponding normal DNA. The individual laboratories classified tumors as MSI if two or more loci were found to be unstable, and if less than two microsatellite loci were found to be unstable, the tumor was classified as MSS. When the same microsatellite locus was examined by more than one group and the interpretation of the banding pattern was contradictory, these results were not included in the total analysis.

Table 1 *List of microsatellite loci studied by the different groups in the first and second part of the study.*

	A. First part of study								B. Second part of study						
Locus	A[a]	B	C	D	E	F	G	Locus	A	B	C	D	E	F	G
								D1S543					x		
D2S123		**X**[b]		**X**			**X**	*D2S123*		**X**	**X**	**X**	**X**	**X**	**X**
D2S134							x	*D2S134*							x
D2S136		x													
D2S177							x	*D2S177*							x
D2S299		x													
								BAT26	**X**			**X**		**X**	
D3S966							x								
D3S1298		x													
D3S1611	x														
D5S82	**X**	**X**					x	*D5S82*	**X**						**X**
D5S107			**X**	**X**				*D5S107*				x			
D5S346	**X**	**X**			**X**	**X**	**X**	*D5S346*	**X**	**X**			**X**	**X**	**X**
								D5S406	x						
								D5S617	x						
								D6S442	x						
								D7S1824					x		
D8S156					x										
								D5S1989	x						
D9S70					x										
D9S156	x														
D9S171						x		*D9S171*						x	
D10S59					x										
D10S89						x		*D10S89, Mfd28*						x	
D10S197						x		*D10S197*							x
								D10S2325					x		
D11S904						x		*D11S904*						**X**	**X**
K-*ras* $(A)_n$	x														
D13S153				x				*D13S153*				x			
D13S175						x									
D13S289	x														
D15S152					x										
D15: 635/636			x												
kard. Aktin, Loc. 15qter				x				kard. Aktin. Loc. 15qter				x			
								D15S230	x						
								D15S643	x						
								HBAP25		x					
TP53(5)$_n$	**X**		**X**		**X**	**X**		*TP53(5)*$_n$						x	
								D17S855					x		
D18S34			**X**	**X**		**X**	**X**	*D18S34, Mfd26*				**X**		**X**	**X**
D18S35		x						*D18S35*		x					
D18S69	x														
								D19S246					x		
								D22S345					x		
								BAT25			**X**			**X**	**X**
								BAT40			x				
Total[c]	8	7	4	5	6	8	7		9	4	3	6	8	9	9

[a] Letters A to G designate the different participants.
[b] The boldface "X" highlights microsatellite markers that have been used by more than one laboratory.
[c] Indicates the total number of loci studied by the respective groups.

Table 2 *Multicenter analysis of 10 patients' samples*

The number of unstable loci in the total number of loci studied in the respective lab is given. LOHs and loci with questionable instability are not included under MSI for the separate labs but are listed additionally. Loci with contradictory results concerning MSI are not included in the total count of all participants taken together.

Patient no.	A[b]	B	C	D	E	F	G	Total	%	MSI status	Immunohistochemistry[a] hMSH2	hMLH1
1	0 of 8, 2 LOHs	1 of 7	0 of 4	0 of 5	0 of 6	0 of 7	0 of 7	1 of 30	3.3	Stable	Pos	Pos
2	4 of 8	1 of 7, 1 ?LOH	0 of 4, 2 LOH	0 of 5, 1LOH	0 of 6	1 of 7	1 of 7, 1 LOH	2 of 30	6.7	Stable	Pos	Pos
3	0 of 8	0 of 7	0 of 4	0 of 5, 1 LOH	0 of 6	0 of 8	0 of 7	0 of 30	0	Stable	Pos	Pos
4	5 of 8	7 of 7	4 of 4	5 of 5	4 of 6	6 of 8	5 of 7, 1 LOH	21 of 31	67.7	Unstable	Pos	Neg
5	2 of 8	0 of 7, 2 ?LOH	0 of 4, 1 LOH	0 of 5	0 of 6	1 of 7, 1 LOH	0 of 7, 1 LOH	1 of 31	3.2	Stable	Pos	Pos
6	9 of 9	3 of 3	3 of 3	5 of 7	6 of 8	9 of 9	9 of 9	27 of 31	87.1	Unstable	Neg	Pos
7	9 of 9	4 of 4	3 of 3	2 of 5, 1 MSI/LOH	7 of 8	5 of 7	7 of 9	27 of 30	90	Unstable	Neg	Pos
8	0 of 9, LOH *D5S82*	0 of 3	0 of 3	0 of 7	1 of 8	0 of 9	0 of 9	1 of 29	3.5	Stable	Pos	Pos
9	1 of 9, LOH *D5S346*	???	0 of 3	0 of 5	0 of 8	0 of 8, LOH *p53*, *D18S34*	0 of 9, LOH *D18S34*	1 of 29	3.5	Stable	Pos	Pos
10	1 of 8, LOH *D5S82*, *D5S346*, *D8S1989*	0 of 4	0 of 3	0 of 6, 1 MSI/LOH	0 of 8	0 of 8	0 of 9, LOH *D5S346*	1 of 30	3.3	Stable	Pos	Pos

[a] Pos, positive; Neg, negative.
[b] Microsatellite instability in the different patients (pt) as assessed in the laboratories A through G.

Results

Initial classification of the tumors as MSI and MSS by the different groups was based on the assignment of tumors with MSI (≥2 unstable loci) or MSS (<2 unstable loci) when the maximum of nine individual loci were considered by each group. In the evaluation of the data from all of the participating laboratories comprising a total of at least 29 microsatellite loci, we reclassified tumors as "low" MSI (≤2 unstable loci) and "high" MSI (>2 unstable loci) to further define microsatellite instability. "High" MSI tumors displayed instability at ≥67.7% of the loci examined as opposed to ≤6.7% for MSS tumors (which contain both "low" MSI and MSS tumors). The retrospective analysis of the 10 patients' DNAs with a defined panel of 10 microsatellite loci, which have been found to be both specific and sensitive in identifying MSI cases (13), confirmed the correct classification of the cases. Moreover, immunohistochemistry showed loss of expression in all high MSI cases (hMSH2 in patients 7 and 8, hMLH1 in patient 5), whereas all low MSI and MSS cases expressed both proteins normally (Table 2). These observations further substantiate the validity of the criteria used in this multicenter study. The mononucleotide markers *BAT25* and *BAT26* were found to have a high predictive value for "high" MSI, which has been described previously (13, 14).

In part I as well as in part II of the study, the patients with "high" MSI of microsatellites were identified accordingly (patients 4, 6, and 7; Table 2). One of the cases appeared completely MSS (patient 3) with the total of 31 loci analyzed. The rest of the cases displayed one or two isolated unstable microsatellite and were, therefore, reclassified as "low" MSI with the total panel of at least 30 loci studied. When the entire panel of loci (29 to 31 total) was examined, the percentage of unstable microsatellite loci, disregarding LOHs, never exceeded 6.7% (patient 2) in the "low" MSI cases. Interestingly, several laboratories that only tested three or four microsatellite loci clearly identified the "high" MSI cases correctly, whereas in the same analysis, they never appeared to detect the "low" MSI tumors. Furthermore, those laboratories that did identify "low" MSI found that many of the singularly MSI-positive microsatellite loci were localized on chromosome 5 (*D5S346* and *D5S82*, patient 2; *D5S406*, patient 9; and *D5S617*, patient 10), and to a lesser extent affected the *D11S904*, *D18S69*, and *D10S2325* microsatellite loci. Interestingly, laboratory A would have inaccurately classified patient 2 as MSI with four unstable loci in a total of eight loci studied (Table 2). However, two of the unstable loci were also studied by other participants and were not found to display MSI. These results exemplify the problem of laboratory evaluation in the determination of MSI.

When comparing the analyses of individual microsatellite loci, discrepancies between the different laboratories became apparent (Table 3). Whereas some microsatellite loci were easily and unambiguously classified (*BAT25*, *D2S123*, *BAT26*, and *D11S904*), the patterns of other microsatellites appeared more difficult to interpret. One of these microsatellite loci (*D5S346*), which was analyzed in the majority of laboratories, produced the most divergent results (Fig. 1 and Table 3). One source of inconsistencies in the interpretation surrounded difficulties in distinguishing between microsatellite instability and LOH. LOH has frequently been found at regions with tumor suppressor genes as *APC*, *Rb*, and others and can sometimes be confounded with MSI, especially if the tissue was not microdissected, and normal cells may have contaminated the tumor tissue, obscuring the band pattern(s). In some instances, it may not be possible to distinguish between MSI and LOH because it is possible that one allele may shift to the exact length of the other allele as a result of MSI so that only one allele is visible in the tumor DNA. Such was the case in patient 5 at locus *D5S346*. Several of the participating institutions interpreted the band pattern as LOH; others classified it as MSI because there was MSI on several other loci. One laboratory called it "MSI or LOH," and one laboratory did not detect any alterations in the band pattern (Fig. 1 and Table 3). The ability to quantify alleles using automated DNA sequencers does not appear to facilitate the differentiation of banding patterns. Furthermore, alternate detection methods do not appear to explain the variability of the results. These data indicate that the choice of microsatellite loci is important for accurate detection of MSI.

Discussion

Microsatellites and MSI have been studied for multiple purposes by geneticists and are being increasingly analyzed in tumor research and clinical laboratories (2). The technology required for microsatellite analysis is well suited for clinical laboratories because no high molecular weight DNA is required. This is an important consideration because microsatellites are as short as 100–200 bp, such that DNA extracted from formalin-fixed and paraffin-embedded tissue can easily be analyzed. Furthermore, the techniques for a PCR-based microsatellite analysis are generally simple and do not require more than routine laboratory equipment plus a thermal cycler and a sequencing gel electrophoresis unit. Working with radiolabeled material is not necessarily required. Therefore, these methods are widely applicable. However, the techniques of microsatellite analysis differ greatly. These differences include variability in the PCR primer panel used to

Table 3 *Analyses of individual microsatellite loci*

Comparison of the results of the microsatellite analyses at identical microsatellite loci performed in more than two different groups (designated A through G as before). *D2S123, Bat26,* and *BAT25* are not shown, as there are no differences in the interpretations of the banding pattern in the different laboratories.

	A	B	C	D	E	F	G	Concordance
Part I[a]								
D5 S346								
Pt. 1	n	n			n	n	n	5/5 100%
Pt. 2	MSI	MSI			n	MSI	MSI	4/5 80%
Pt. 3	n	n			n	n	n	5/5 100%
Pt. 4	MSI	MSI			n	MSI	n	3/5 60%
Pt. 5	MSI	LOH /MSI			n	LOH	LOH	4/5 80%
D18S34								
Pt. 1			n	n		n	n	4/4 100%
Pt. 2			n	n		n	n	4/4 100%
Pt. 3			n	LOH		n	n	3/4 75%
Pt. 4			MSI	MSI		MSI	MSI	4/4 100%
Pt. 5			n	n		ND	n	3/3 100%
D5S82								
Pt. 1	n	n					n	3/3 100%
Pt. 2	MSI	LOH?					LOH	3/3 100%
Pt. 3	n	n					n	3/3 100%
Pt. 4	MSI	MSI					LOH	3/3 100%
Pt. 5	n	n					n	3/3 100%
Part II								
D5 S346								
Pt. 6	MSI	ND			MSI	MSI	MSI	4/4 100%
Pt. 7	MSI	MSI			MSI	MSI	MSI	5/5 100%
Pt. 8	n	n			n	n	n	5/5 100%
Pt. 9	LOH	n			n	n	n	4/5 80%
Pt. 10	LOH	n			n	n	LOH	3/5 60%
D18S34								
Pt. 6				MSI		MSI	MSI	3/3 100%
Pt. 7				MSI		ND	MSI	2/2 100%
Pt. 8				LOH		n	n	2/3 66%
Pt. 9				LOH		LOH	LOH	3/3 100%
Pt. 10				n		n	n	3/3 100%

[a] Pt., patient; ND, not done; n, normal.

amplify the microsatellite sequences as well as different methods of visualization of the PCR products.

To determine the source of potential discrepancies in the techniques of microsatellite analysis that might be applied to clinical diagnostic laboratories, the molecular biology section of the HNPCC Study Group–Germany performed a prospective comparison of microsatellite methodological approaches performed by several laboratories to a total of 10 pairs of normal and tumor colorectal DNAs. One of the goals of this study was to assess the minimal number of microsatellite loci that are required for quality analysis.

Although the classification of tumors as unstable or stable was concordantly achieved, the analysis of identical microsatellite loci in different laboratories varied, especially in the first part of the study. Furthermore, distinguishing between LOH and MSI appeared to be difficult and even impossible in some cases. However, if a tumor displays MSI at several loci, it is highly likely that an interpretation of LOH at a questionable loci might indeed be MSI. Typically, tumors with MSI do not display the gross chromosomal aberrations and LOHs that characterize the appellation "suppressor pathway" of carcinogenesis, which has led to their designation as resulting from the "mutator pathway" (15). On the other hand, if there is no MSI at other loci, the probability of an LOH at a questionable locus would be high (especially when the locus is in the region of a known or putative tumor suppressor gene). The use of an automated sequencer may allow a differential interpretation that is based on the quantitation of bands. However, this differentiation is only applicable if identical amounts of DNA were amplified in the PCR reactions using tumor and normal DNA and the tumor DNA is not contaminated with normal tissue. In our study, differences in the interpretation of the banding pattern could not be explained with different detection methods. Regardless of the use of these rigorous techniques, there will be tumor samples where no final decision can be established. In most cases, the diagnostic problems at single loci will not influence the classification of a tumor as "high" MSI as long as sufficient microsatellite loci are studied. Further widespread clinical agreement on a defined microsatellite panel may also help in this regard (13).

There is disagreement about the minimal number of microsatellite loci to be studied and how many loci have to display instability to classify a tumor as MSI. A number of publications have classified tumors as MSI when as few as one of two loci appeared unstable (16). A more rigorous definition of MSI demands at least two unstable loci or instability in greater than 10% of at least seven studied microsatellite loci (17–19). A measure of MSI classification can be garnered from patient 2 in our study, where MSI was found in 2 of 30 loci. Considering the high number of total loci studied, these two loci only account for 6.7% of the microsatellite loci and would thus not be regarded as MSI. This case underlines the observation that where multiple microsatellite loci are analyzed, the percentage of unstable loci is decisive in determining MSI as opposed to an absolute number of microsatellite loci. A similar conclusion was drawn in a study of 17 Barrett's-associated esophageal adenocarcinomas, where at least 128 microsatellite loci were analyzed (20). Not a single case was found to be absolutely free of MSI. However, the authors distinguished between a low level of MSI in 1–10 loci (0.8–8.2% of loci studied) and one case with high instability in 58 of 128 loci (45.3%) Interestingly, no clinicopathological differences could be demonstrated between these two groups. Furthermore, tetranucleotide instability was found more often in cases with low level MSI. This difference was attributed to a higher baseline mutation rate in tetranucleotide repeats (21), which may have had a special impact on MSI analysis in that study, because 82 (59%) tetranucleotide markers were used. Another study of primary and metastatic colorectal cancers makes a distinction between MSI in at least one of seven *versus* at least two of seven microsatellite loci (22). The cases with MSI at ≥2 loci clinicopathologically resemble HNPCC, whereas cases with microsatellite instability in only one locus cannot be distinguished clinicopathologically from other sporadic tumors with MSS. These authors additionally observe a higher number of MSI in dinucleotide repeats compared to pentanucleotide repeats. It is important to note that the mismatch repair genes have not been analyzed in either of these two studies.

The cases analyzed in this study have been examined by immunostaining for the tissue expression of hMSH2 and hMLH1. Interestingly, "high MSI" is accompanied by loss of expression of either hMSH2 or hMLH1, whereas in tumors classified as MSS or "low MSI," both mismatch repair proteins are always detectable. This finding underlines the importance of a rigorous definition of MSI for

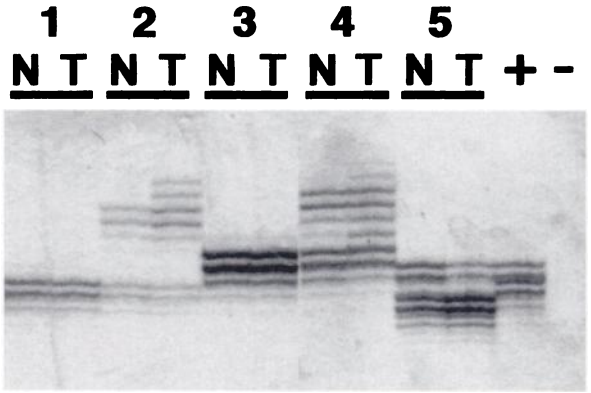

Fig. 1. Microsatellite analysis with the *D5S346* marker visualized by silver nitrate staining. Patients 2 and 4 display MSI. In patient 5, differentiation between MSI and LOH was not possible. +, positive control; −, negative control without template DNA.

the diagnostic relevance for cases with mismatch repair defects, (although the presence of mutations in the genes still needs to be confirmed). Recently, it has been shown that the microsatellite marker *BAT26* is a sensitive marker of MSI, even if studied as the one and only locus (13, 14). Our study confirms that *BAT26* was unstable in all of the "high" MSI tumors; however, high MSI tumors have been described that do not display instability at the *BAT26* locus (13).

MSI at multiple loci has been shown to result from defects in the mismatch repair genes in colorectal carcinomas and other tumors related to the HNPCC syndrome (5, 23, 24). However, the cause of MSI at isolated loci is still unknown. There is a suggestion that some background instability of microsatellites exists that depends on the repeat type (21, 25).

Based on our results, we propose that five well-defined microsatellite loci should be analyzed in the first run and five additional microsatellite loci should be added in cases where less than two loci display MSI. The microsatellite panel should comprise different repeat types. In addition to dinucleotide repeats, mononucleotide repeats appear extremely informative and easy to interpret (13). The number of loci analyzed and the number of unstable loci should always be indicated to make a comparison of data from different laboratories possible. In the samples presented here, the percentage of loci with MSI was either $\leq$6.7% or $\geq$67.7%, which allowed an easy determination of the microsatellite status. The HNPCC study group–Germany proposes that MSI should only be diagnosed if >20% of loci studied display alterations of the band pattern in a random panel of microsatellite markers with different repeat lengths. With this rigorous definition, MSI becomes a reliable method to assess the molecular characteristics of tumors and to recommend therapeutic strategies.

References

1. Tautz, D. Notes on the definition and nomenclature of tandemly repetitive DNA sequences. Exper. Suppl. (Basel), *67:* 21–28, 1993.
2. Ruschoff, J., Bocker, T., Schlegel, J., Stumm, G., and Hofstaedter, F. Microsatellite instability: new aspects in the carcinogenesis of colorectal carcinomas. Virchow Arch., *426:* 215–222, 1995.
3. Schlegel, J., Bocker, T., Zirngibl, H., Hofstaedter, F., and Ruschoff, J. Detection of microsatellite instability in human colorectal carcinomas using a non-radioactive PCR-based screening technique. Virchow Arch., *426:* 223–227, 1995.
4. Canzian, F., Salovaara, R., Hemminki, A., Kristo, P., Chadwick, R. B., Aaltonen, L. A., and de la Chapelle, A. Semiautomated assessment of loss of heterozygosity and replication error in tumors. Cancer Res., *56:* 3331–3337, 1996.
5. Aaltonen, L. A., Peltomaki, P., Leach, F. S., Sistonen, P., Pylkkaenen, L., Mecklin, J. P., Jaervinen, H., Powell, S. M., Jen, J., Hamilton, S. R., Peterson, G. M., Kinzler, K. W., Vogelstein, B., and de la Chapelle, A. Clues to the pathogenesis of familial colorectal cancer. Science (Washington DC), *260:* 812–816, 1993.
6. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D., and Perucho, M. Ubiquitous somatic mutations in simple repetitive sequences reveal a new mechanism for colonic carcinogenesis. Nature (Lond.), *363:* 558–556, 1993.
7. Thibodeau, S. N., Bren, G., and Schaid, D. Microsatellite instability in cancer of the proximal colon. Science (Washington DC), *260:* 816–819, 1993.
8. Samowitz, W. S., Slattery, M. L., and Kerber, R. A. Microsatellite instability in human colonic cancer is not a useful indicator of familial colorectal cancer. Gastroenterology, *109:* 1765–1771, 1995.
9. Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E., and Inouye, M. Frameshift mutations and the genetic code. Cold Spring Harbor Symp. Quant. Biol., *31:* 77–84, 1966.
10. Kunkel, T. A. Slippery DNA and diseases. Nature (Lond.), *365:* 207–208, 1993.
11. Sambrook, J., Fritsch, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual, Ed. 2. 6.34–6.35. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.
12. Bocker, T., Schlegel, J., Kullmann, F., Stumm, G., Zirngibl, H., Epplen, J. T., and Rueschoff, J. Genomic instability in colorectal carcinomas: comparison of different evaluation methods and their biological significance. J. Pathol., *179:* 15–19, 1996.
13. Dietmaier, W., Wallinger, S., Bocker, T., Kullmann, F., Fishel, R., and Rüschoff, J. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res., *57:* 4749–4756, 1997.
14. Hoang, J. M., Cottu, P. H., Thuille, B., Salmon, R. J., Thomas, G., and Hamelin, R. BAT-26, an indicator of the replication error phenotype in colorectal cancers and cell lines. Cancer Res., *57:* 300–303, 1997.
15. Perucho, M. Cancer of the microsatellite mutator phenotype. J. Biol. Chem., *377:* 675–684, 1996.
16. Chong, J. M., Fukuyama, M., Hayashi, Y., Takizawa, T., Koike, M., Konishi, M., Kikuchi-Yanoshita, R., and Miyaki, M. Microsatellite instability in the progression of gastric carcinoma. Cancer Res., *54:* 4595–4597, 1994.
17. Lothe, R. A., Peltomaki, P., Meling, G. I., Aaltonen, L. A., Nystroem-Lahti, M., Pylkkaenen, L., Heimdal, K., Andersen, T. I., Moller, P., Rognum, T. O., Fossa, S. D., Haldorsen, T., Langmark, F., Brogger, A., de la Chapelle, A., and Borresen, A. L. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. Cancer Res., *53:* 5849–5852, 1993.
18. Peltomaki, P., Aaltonen, L. A., Sistonen, P., Pylkkaenen, L., Mecklin, J. P., Jaervinen, H., Green, J. S., Jass, J. R., Weber, J. L., Leach, F. S., Petersen, G. M., Hamilton, S. R., de la Chapelle, A., and Vogelstein, B. Genetic mapping a locus predisposing to human colorectal cancer. Science (Washington DC), *260:* 810–812, 1993.
19. Risinger, J. I., Berchuck, A., Kohler, M. F., Watson, P., Lynch, H. T., and Boyd, J. Genetic instability of microsatellites in endometrial carcinoma. Cancer Res., *53:* 5100–5103, 1993.
20. Gleeson, C. M., Sloan, J. M., McGuigan, J. A., Ritchie, A. J., Weber, J. L., and Russel, S. E. H. Ubiquitous somatic alterations at microsatellite alleles occur infrequently in Barrett's-associated esophageal adenocarcinoma. Cancer Res., *56:* 259–263, 1996.
21. Weber, J. L., and Wong, C. Mutation of human short tandem repeats. Hum. Mol. Genet., *2:* 1123–1128, 1993.
22. Ishimaru, G., Adachi, J. I., Shiseki, M., Yamaguchi, N., Muto, T., and Yokata, J. Microsatellite instability in primary and metastatic colorectal cancers. Int. J. Cancer, *64:* 153–157, 1995.
23. Fishel, R., Lescoe, M. K., Rao, M. R. S., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. The human mismatch repair gene homologue *MSH2* and its association with hereditary nonpolyposis colon cancer. Cell, *75:* 1027–1038, 1993.
24. Leach, F. S., Nicolaides, N. C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomaki, P., Sistonen, P., Aaltonen, L. A., Nystroem-Lahti, M., Guan, X. Y., Zhang, J., Meltzer, P. S., Yu, J. W., Kao, F. T., Chen, D. J., Cerasoletti, K. M., Fournier, R. E. K., Todd, S., Lewis, T., Leach, R. J., Naylor, S. L., Weissenbach, J., Mecklin, J. P., Jaervinen, H., Petersen, G. M., Hamilton, S. R., Green, J., Jass, J., Watson, P., Lynch, H. T., Trent, J. M., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. Mutations of a *MutS* homolog in hereditary nonpolyposis colorectal cancer. Cell, *75:* 1215–1225, 1993.
25. Mahtani, M. M., and Willard, H. F. A polymorphic X-linked tetranucleotide repeat locus displaying a high rate of new mutation: implications for mechanisms of mutation at short tandem repeat loci. Hum. Mol. Genet., *2:* 431–437, 1993.