LETTER TO JMG

A genotype-phenotype correlation in HNPCC: strong predominance of *msh2* mutations in 41 patients with Muir-Torre syndrome

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Muir-Torre syndrome (MTS; MIM 158320) is an autosomal dominant predisposition to skin tumours and various internal malignancies. Clinical criteria for a diagnosis of MTS are the synchronous or metachronous occurrence of at least one sebaceous gland neoplasia and at least one internal neoplasm in a patient (regardless of the family history).[1 2] The sebaceous gland neoplasias comprise adenomas, epitheliomas (sebaceomas), and carcinomas. In contrast, the frequent sebaceous gland hyperplasia is not indicative of MTS.[2 3] According to Schwartz and Torre,[2] the sebaceous neoplasias precede the internal neoplasias or are concurrent with them in 41% of MTS patients. As sebaceous gland neoplasias are rare, MTS should always be suspected when a sebaceous tumour has been diagnosed. Cystic sebaceous neoplasia is probably the most sensitive marker for this tumour predisposition syndrome.[2 4–6] Colorectal cancer is by far the most common internal malignancy in MTS patients.[7] The spectrum of internal malignancies in MTS is similar to the various tumour entities observed in hereditary non-polyposis colorectal cancer (HNPCC; MIM 114500). HNPCC is an autosomal dominant cancer predisposition syndrome characterised by early onset of colorectal cancer and other associated tumours.[8 9] Several genes underlying HNPCC which are involved in DNA mismatch repair (MMR) have been identified within the last decade.[10–13] Germline mutations in the DNA MMR genes were detected in a high proportion of MTS patients, demonstrating that MTS most often represents a phenotypic variant of HNPCC.[14 15] Due to the underlying genetic mechanisms of tumourigenesis, tumours of these MTS patients exhibit high microsatellite instability (MSI-H), the characteristic feature of HNPCC tumours.[16] Microsatellite analysis in tumour tissue of MTS patients therefore provides a useful tool to pre-select patients for mutation analysis in DNA MMR genes.[3] Immunohistochemical testing for expression of the MSH2 and MLH1 proteins in skin tumour tissue is an alternative reliable screening method with high predictive value for the diagnosis of DNA mismatch repair deficient MTS (HNPCC).[17 18]

A diagnosis of HNPCC in an MTS patient is of major importance for both the patient and his/her close relatives, as all family members who inherited the DNA MMR defect have a substantially higher risk for HNPCC malignancies and should therefore undergo regular cancer surveillance examinations. Identification of the underlying DNA MMR germline mutation in the index patient enables predictive genetic testing of his/her family members at risk.

To date, DNA MMR gene mutations in MTS patients have been reported in both the *MSH2* and *MLH1* genes. While the proportions of *MLH1* and *MSH2* mutations in HNPCC are almost equal (ICG-HNPCC mutation database, http://www.nfdht.nl), in MTS the vast majority of mutations have been identified in *MSH2*. This suggests a genotype correlation for the Muir-Torre phenotype among HNPCC patients.[15]

The aim of this study was to further support this genotype–phenotype correlation in HNPCC. We determined the DNA MMR mutation spectrum in a large MTS patient sample after pre-selection by examination for MSI and immunostaining in tumour tissue. For this purpose we extended our previously reported sample of 15 MTS patients[4 15 19 20] to a total of 41

Key points

- Sebaceous gland neoplasms are the characteristic cutaneous manifestation of Muir-Torre syndrome (MTS), a phenotypic variant of hereditary non-polyposis colorectal cancer (HNPCC).
- We performed mutation analysis in 41 unrelated index patients diagnosed with Muir-Torre syndrome or a sebaceous neoplasm. Thirty seven of these patients had been pre-selected for DNA mismatch repair deficiency in tumour tissue following proof of either high microsatellite instability or loss of MSH2/MLH1 protein expression, or both. In 27 of the 41 patients, we detected germline mutations in the DNA mismatch repair genes *MSH2* and *MLH1* (mutation detection rate of 66%); three mutations were large genomic deletions.
- In contrast to HNPCC patients without the MTS phenotype, significantly more *MSH2* mutations were detected among the MTS patients: 25 (93%) mutations were located in *MSH2* compared to only two in *MLH1*. Our findings should have consequences for mutation detection protocols in MTS patients or HNPCC patients who have family members with MTS.
- Interestingly, six (22%) of the mutation carriers do not meet the Bethesda criteria for HNPCC, nor do their families. Applying only the current Bethesda criteria would have resulted in these patients being overlooked. The Muir-Torre phenotype should therefore be regarded as a highly specific indicator for hereditary DNA mismatch repair deficiency. Even in patients not meeting the Bethesda criteria, a sebaceous neoplasm makes diagnosis of HNPCC likely.

Abbreviations: DHPLC, denaturing high performance liquid chromatography; HNPCC, hereditary non-polyposis colorectal cancer; MLPA, multiplex ligation-dependent probe amplification; MMR, mismatch repair; MSI-H, high microsatellite instability; MTS, Muir-Torre syndrome; PTT, protein truncation test; SSCP, single strand conformation polymorphism

Table 1 Tumour history of 26 newly identified patients and their families

Patient number	Patient's history	Family history
398* (MTS-24)	Keratoacanthoma (50 years), sebaceous gland hyperplasia (55 years), sebaceous adenoma (57 years), squamous cell carcinoma (57 years), sebaceous gland hyperplasia (61 years), squamous cell carcinoma (66 years), transitional cell carcinoma (ureter) (66 years), carcinoma of jejunum (66 years)	Mother: uterine cancer (46 years), small intestinal cancer (50 years), unknown abdominal cancer (86 years); brother of mother: breast cancer (50 years)
512	Colon carcinoma (50 years), squamous cell carcinoma (58), bladder cancer (58 years), multiple sebaceous adenomas (58 years)	No HNPCC related tumours
527	Sebaceous carcinoma (60 years)	Mother: colorectal cancer (40 years), bladder cancer (41 years); brother of mother and his son: gastric cancer (55 years, 39 years)
535*	Small bowel carcinoma (34 years), sebaceous adenoma (47 years)	Mother: colorectal cancer (51 years)
554* (MTS-21)	Cystic sebaceous tumour (58 years)	Sister: endometrial cancer (39 years); father: gastric cancer (<60 years)
555* (MTS-23)	Colon carcinoma (34 years), breast cancer (46 years), uterine cancer (47 years), colon carcinoma (50 years), colon carcinoma (59 years), cystic sebaceous tumour (66 years)	Mother: colon cancer (age unknown); three siblings of mother: gastric cancer (ages unknown); sister of mother: breast cancer (age unknown); cousin: renal cancer (age unknown)
593*	Rectal carcinoma (34 years), sebaceous epithelioma (57 years), sebaceous carcinoma (58 years)	Brother of mother: pancreatic cancer (74 years); grandmother: bladder or liver carcinoma (age unknown)
626	Multiple sebaceous adenomas (<53 years), sebaceous carcinoma (60 years), colon carcinoma (62 years)	No tumours reported
628* (ST-27)	Colon carcinoma (60 years), sebaceous carcinoma (64 years)	Mother: cervical cancer (age unknown)
631* (MTS-18)	Urothelial carcinoma (56 years), multiple sebaceous tumours and keratoacanthomas (57 years)	Brother: colorectal cancer (age unknown)
655* (ST-29)	Multiple colon carcinomas (age unknown), multiple sebaceous tumours (age unknown), keratoacanthoma (67 years)	Brother: colorectal cancer (age unknown); father: colorectal cancer (age unknown)
667* (ST-28)	Urothelial carcinoma (60 years), bladder cancer (63 years), squamous cell carcinoma (65 years), sebaceous epithelioma (65 years)	Mother: colon carcinoma (78 years); sister of mother: bladder cancer (80 years)
684 (ST-9)	Colon carcinoma (35 years), cystic sebaceous tumour (48 years)	Brother of father: colon carcinoma (72 years)
712	Colon carcinoma (42 years), breast cancer (49 years), colon carcinoma (54 years), sebaceous carcinoma (57 years)	Sister: colon carcinoma (42 years); mother: colon carcinoma (40 years)
726*	Sebaceous adenoma (60 years), colorectal and gastric cancer (<62 years)	No family history available
727	Colon carcinoma (50 years), gastric cancer (68 years), sebaceous hyperplasia and sebaceous carcinoma (70 years)	Several family members with colon carcinoma (ages unknown)
747 (ST-31)	Colorectal cancer (44 years), bladder cancer (70 years), sebaceous carcinoma (70 years), sebaceous adenoma (71 years), basal cell carcinomas (>57 years)	Brother: colorectal cancer (age unknown); mother and seven siblings of mother: colorectal cancer or other malignancies (ages unknown)
762*	Endometrial carcinoma (30 years), two colorectal cancers (54 years), desmoid (jejunum) (56 years), sebaceous adenoma (60 years)	Sister: endometrial cancer (41 years), colon carcinoma (51 years); brother of mother: gastric carcinoma (36 years)
765* (ST-63)	Cystic sebaceous tumour (61 years), bladder cancer (64 years), colon carcinoma (68 years) (further history unknown)	Mother: unknown carcinoma of the lower abdomen (67 years)
784 (ST-24)	Sebaceous epithelioma (62 years)	No tumours reported
785 (ST-34)	Sebaceous epithelioma (86 years)	Father: colon carcinoma (age unknown)
787 (ST-10)	Colorectal cancer (72 years), cystic sebaceous tumour (79 years)	No tumours reported
788* (ST-33)	Colon carcinoma (54 years), squamous cell carcinoma, sebaceous epithelioma and two sebaceous adenomas (64 years)	Mother: colon carcinoma (48 years), unknown abdominal carcinoma (53 years); sister of mother: colorectal cancer (48 years); cousin: breast cancer (59 years)
808 (ST-39)	Colorectal cancer (48 years), unknown abdominal cancer (67 years), two sebaceous epithelioma (78 years)	Mother: unknown abdominal cancer (48 years); brother of mother: lung cancer (age unknown)
810*	Multiple sebaceous hyperplasias, keratoacanthomas, and urothelial cancer (52 years), bladder cancer (53 years), two colon carcinomas (54 years)	Father: colon carcinoma (49 years)
852 (ST-41)	Colon cancer (74 years), sebaceous epithelioma and basal cell carcinoma (81 years), two basal cell carcinomas (age unknown)	No tumours reported

The 26 newly identified patients were diagnosed with MTS or suspected of MTS and were included in mutation analysis.
*A germline mutation was detected in these patients.

patients. Either the patients were diagnosed with MTS by clinical criteria or MTS was suspected due to the occurrence of at least one sebaceous neoplasm. To our knowledge, this is the largest sample of MTS patients screened for DNA MMR mutations to date.

METHODS

Selection of patients included in mutation screening

Screening for germline mutations in *MSH2* and *MLH1* was performed in 41 unrelated index patients. Of the 41 patients, 37 met the diagnostic criteria for MTS. In four patients, only one sebaceous neoplasm had been diagnosed, raising the suspicion of MTS.

Of the 41 index patients, 40 originated from a sample of patients who had been ascertained on the basis of both a sebaceous skin neoplasm and an internal malignancy, or on a sebaceous skin neoplasm only. Ascertainment of these patients had been carried out irrespective of family history or age at onset of tumours. Only one index patient (patient 62) is a member of a known HNPCC family which was originally ascertained following early manifestation of multiple colorectal cancers.

Pre-screening analysis of sebaceous tumour tissue for MSI-H and for loss of MLH1 and MSH2 protein expression was performed as previously reported.[18] In 37 patients, an underlying DNA MMR gene defect was indicated by tumour tissue analysis, either microsatellite analysis or immunohistochemistry, or both. These 37 patients and a further four patients, in whom a clinical diagnosis of MTS had been made but for whom no tumour tissue was available, were included

Table 2 Mutations detected in 27 index MTS patients

Patient number	Gene	Exon	Mutation	Effect	MSI status	MMR protein loss at IHC
555 (MTS-23)	*MSH2*	1 to 6	Deletion of exons 1–6	Large deletion	MSI-H	ND
554 (MTS-21)	*MSH2*	1	c.145delG†	Frameshift	MSI-H	ND
130	*MSH2*	2	c.289_290ins22bp* (c.268–289dup)	Frameshift	MSI-H	MSH2
593	*MSH2*	2	c.289C>T	Q97X	NT	NT
435 (MTS-10)	*MSH2*	3	c.380_381delAT*	Frameshift	MSI-H	NT
162	*MSH2*	3	c.478C>T†	Q160X	MSI-H	MSH2
726 (ST-62)	*MSH2*	3	c.592_593insG†	Frameshift	ND	ND
278	*MSH2*	5	c.862C>T*	Q288X	MSI-H	MSH2
122	*MSH2*	5	c.942+3 A>T	Alteration of splicing	MSI-H	NT
765 (ST-63)	*MSH2*	7	c.1165C>T	R389X	ND	MSH2
535	*MSH2*	7	c.1189C>T	Q397X	MSI-H	MSH2
788 (ST-33)	*MSH2*	8	c.1373T>G	L458X	ND	MSH2
631 (MTS-18)	*MSH2*	9, 10	Deletion of exons 9, 10†	Large deletion	MSI-H	NT
810	*MSH2*	10	c.1571G>C	R524P	MSI-H	None
MTS-2a	*MSH2*	10	c.1576delA*	Frameshift	MSI-H	ND
398 (MTS-24)	*MSH2*	10	c.1578delC†	Frameshift	MSI-H	MSH2
132	*MSH2*	11	c.1676delA*	Frameshift	MSI-H	NT
167	*MSH2*	11	c.1700_1704delAAACA*	Frameshift	MSI-H	MSH2
133	*MSH2*	12	c.1809delT*	Frameshift	MSI-H	MSH2
655 (ST-29)	*MSH2*	12	c.2005+2T>C†	Alteration of splicing	ND	ND
851 (MTS-8)	*MSH2*	13	c.2015delT*	Frameshift	MSI-H	NT
62	*MSH2*	13	c.2090 G>T*	C697F	MSI-H	MSH2
667 (ST-28)	*MSH2*	13	c.2131C>T	R711X	MSI-H	MSH2
628 (ST-27)	*MSH2*	14	c.2228C>G	S743X	MSI-H	MSH2
762	*MSH2*	15, 16	Deletion of exons 15, 16†	Large deletion	MSI-H	MSH2
MTS-9	*MLH1*	2	c.150_151insT*	Frameshift	MSI-H	ND
MTS-14	*MLH1*	16	c.1884_1888delGGAAA*	Frameshift	MSI-H	ND

*Mutation (according to current nomenclature) in this patient diagnosed with MTS or suspected MTS was reported previously[4 15 19 20]; †novel mutation not listed in the ICG-HNPCC database (http://www.nfdht.nl). IHC, immunohistochemistry; ND, not done; NT, no tumour tissue available.

in the mutation screening. Fifteen of these index patients were described previously,[4 15 19 20] while 26 patients are reported here for the first time (table 1).

Written informed consent was obtained from all patients included. The study was approved by the ethical committees of the University Hospitals in Duesseldorf and Bonn.

Search for germline mutations in *MSH2* and *MLH1*

Peripheral blood was drawn from all index patients to extract genomic DNA by a standard salting out procedure.[21] The search for germline mutations in the previously reported patients had been performed by using the protein truncation test (PTT), heteroduplex analysis, or single strand conformation polymorphism (SSCP) analysis according to Kruse *et al*[15] followed by direct sequencing (using an ABI 377 sequencer; Applied Biosystems, Weiterstadt, Germany). In the newly admitted 26 patients and in three of the previously reported patients (patients 122, 162, and 199) in whom no mutation had been identified, denaturing high performance liquid chromatography (DHPLC) was applied, as described by Holinski-Feder *et al*,[22] followed by direct sequencing. For detection of large genomic deletions we applied multiplex ligation-dependent probe amplification (MLPA) according to the manufacturers' protocol (MRC-Holland, Amsterdam, The Netherlands).

Statistical analysis

Fisher's exact test was applied in order to test the null hypothesis of no difference in *MSH2* and *MLH1* mutation frequency between patients with the MTS phenotype and HNPCC patients without the MTS phenotype.

RESULTS

We searched for a germline mutation in *MSH2* and *MLH1* in 41 patients either diagnosed with MTS according to the clinical criteria or with suspicion of MTS due to the occurrence of a sebaceous neoplasia. A total of 37 of these patients had been pre-selected by examination of tumour tissue for loss of DNA MMR protein expression and/or high microsatellite instability. In 27 of the 41 patients, germline mutations were identified, corresponding to a mutation detection rate of 66%. Sixteen of these mutations are described in the context of Muir-Torre syndrome for the first time in this study; seven mutations are novel and not listed in the ICG-HNPCC database (table 2).

A total of 24 mutations were identified in the 37 patients pre-selected by tumour tissue analysis. The tumour tissue of *MSH2* mutation carriers was available for immunohistochemistry in 14 patients. In 13 patients, the results of immunohistochemical analysis indicated localisation of a mutation in the *MSH2* gene. Only in one patient (patient 810) was neither loss of MSH2 nor loss of MLH1 expression found; this patient carried a missense variant in *MSH2*. A mutation was detected in three out of four patients with MTS from whom no tumour tissue was available for pre-screening.

Twenty five (93%) of the identified mutations are predicted to lead to a truncated protein and therefore have to be regarded as definitely disease-causing genetic alterations. The majority of these are small point mutations: a total of 12 frameshift mutations, eight nonsense mutations, and two alterations at the highly conserved splice site positions were identified. Three large genomic deletions encompassing several exons were detected by additional deletion screening.

Two *MSH2* missense mutations of unknown relevance were identified, R524P (patient 810) and C697F (patient 62). No other possible disease-causing variants in *MSH2* or *MLH1* were found in these patients. In family 62, DNA samples of four affected family members from two generations were analysed for the missense mutation and all affected family members were found to be carriers of variant C697F. Immunohistochemical tissue analysis in patient 62 revealed loss of MSH2 expression. This finding further supports the assumption that variant C697F is indeed disease causing. As regards patient 810, no other affected family members were available for segregation analysis and immunohistochemistry

showed no DNA MMR protein loss. However, the tumour tissue was reported to be MSI-H.

The majority of the 27 germline mutations were located in the *MSH2* gene (25/27 = 93%) and only two mutations (7%) were identified in *MLH1*. This proportion of *MSH2* mutations was much higher than in a large sample of 105 mutation carriers with the HNPCC phenotype (without MTS). Among these 105 patients, 54 (51%) *MSH2* mutations and 51 (49%) *MLH1* mutations were detected (unpublished own observation). The overrepresentation of *MSH2* mutations in patients with the MTS phenotype compared to HNPCC patients without the MTS phenotype is highly significant ($p<0.001$; two-tailed Fisher's exact test).

The *MSH2* mutations were distributed over the whole *MSH2* gene. Approximately 10% of the *MSH2* mutations were large genomic deletions.

A total of 40 index patients had originally been ascertained on the basis of an MTS or a sebaceous neoplasm, only patient 62 being from a previously known HNPCC family. The personal history of all 27 mutation carriers was available, as was the family history of most of the mutation carriers. Fifteen of the 27 mutation carriers met the Bethesda criteria for HNPCC (see comments in Rodriguez-Bigas *et al*[23]). The family history of the four patients who did not meet the Bethesda criteria would have led to a suspicion of HNPCC. Eight of the identified mutation carriers did not meet the Bethesda guidelines for HNPCC. In six out of these eight patients, neither the family nor individual relatives met any of the criteria of the Bethesda guidelines. In two of these eight patients, no family history was available.

DISCUSSION

MTS is a rare autosomal dominant disorder predisposing to sebaceous skin neoplasms and internal malignancies. A subgroup of MTS represents an allelic variant of HNPCC. DNA MMR gene defects, the genetic alterations underlying HNPCC, have been reported in this subgroup of MTS. Accordingly, affected family members of MTS patients may manifest characteristic HNPCC tumours with or without cutaneous tumours typical for MTS.

We performed screening for germline mutations in *MSH2* and *MLH1* genes in 41 unrelated index patients diagnosed with MTS or suspected of MTS, most of them pre-selected for MMR deficiency in their tumour tissue. We identified 27 germline mutations in the DNA MMR genes *MSH2* and *MLH1*, 93% of these mutations being located in *MSH2*. While in ''pure'' HNPCC patients (HNPCC patients without the MTS phenotype) the ratio of mutations in *MSH2* and *MLH1* was about 1:1 (unpublished own observation and Papadopoulos and Lindblom[24]), the proportion of *MSH2* mutations was significantly higher in the MTS patients reported in this study.

Indications that *MSH2* is the causative gene in most of the MTS patients have been previously described in the literature: there are several case reports or reports on small MTS patient samples presenting an *MSH2* mutation.[14 25–27] In our previously reported 11 mutation carriers, *MSH2* was found to be the causative gene in nine cases.[4 15 20] Other authors reported a total of 11 different *MSH2* mutations in MTS patients. In contrast, only three different *MLH1* mutations have been reported in MTS patients so far, and two of these are part of our MTS sample.[4 19 28]

We did not examine the *MSH6* gene in the 14 patients in whom no mutation in the *MSH2* or *MLH1* genes was detected. In 11 of these 14 patients, immunohistochemical examination of tumour tissues was performed, and loss of MSH2 or MLH1 expression was found in six and four cases, respectively. One tumour showed weak expression of both MSH2 and MLH1, although evaluation of this tumour remains questionable. It has been demonstrated that the vast majority of tumours in patients with a germline mutation in the *MSH6* gene show normal MSH2 and MLH1 staining.[29 30] Therefore, it is very unlikely for as many as 10 of the 14 patients to exhibit an *MSH6* germline mutation.

To date, few other genotype–phenotype correlations have been reported for *MSH2* mutation carriers. The lifetime risk of developing cancer at any site or in the urinary tract has been reported to be significantly higher for *MSH2* mutation carriers than for *MLH1* mutation carriers.[31] According to the same study, the risk of developing cancer of the colorectum, endometrium, ovaries, stomach, and brain was also higher in *MSH2* mutation carriers. However, this difference was not significant.

Literature data also point towards mutations in *MSH6* resulting in a different phenotype compared to mutations in *MSH2* and *MLH1*. A high proportion of *MSH6* germline mutations were identified in atypical HNPCC families with a high frequency of atypical hyperplastic lesions and carcinomas of the endometrium.[32] To date, no mutations in the *MSH6* gene have been reported in patients with the MTS phenotype.

The *MSH2* mutations detected in our MTS sample were evenly distributed over the whole length of the gene, an observation that is also made in HNPCC patients (ICG-HNPCC mutation database and own unpublished observation). Each of the mutations occurred only once in our index patients. This finding may be due to the sample size and does not contradict the findings in large HNPCC patient samples, where some mutations were identified more than once in unrelated patients. However, classic mutational hot spots do not exist.

No specific mutations or certain *MSH2* domains are correlated with the MTS phenotype. Additionally, a large proportion of the mutations detected in our MTS patients were also previously described in HNPCC patients or families without the MTS phenotype. Obviously, *MSH2* mutation carriers are at a much higher risk of developing a sebaceous neoplasm compared to *MLH1* mutation carriers. However, the overall incidence of the rare sebaceous neoplasms compared to other HNPCC malignancies seems to be quite low among *MSH2* mutation carriers. This assumption is further supported by the observation that in many *MSH2* mutation carriers from the same family, frequently only a single family member is affected with sebaceous tumours.

The overall mutation detection rate of 66% and the detection of large genomic deletions in 7% of our MTS patients are both in line with the mutation and deletion detection rates in a large sample of HNPCC patients (61% and 10.6%, respectively) recently found by our group.[33]

Our finding of a clear-cut genotype–phenotype correlation has consequences for mutation screening in MTS patients or HNPCC index patients with reported MTS patients among their relatives. As the chance of finding an *MSH2* mutation in these patients is relatively high, mutation screening should start with the *MSH2* gene, unless immunohistochemical results of the patients' tumour tissue are available and point towards another causative DNA MMR gene. A search for large genomic deletions should be included in the mutation screening protocols for MTS patients.

The Bethesda guidelines for HNPCC list a number of tumours as specific malignancies for HNPCC. However, sebaceous neoplasms, the typical tumours of the MTS phenotype, are not mentioned as indicators for HNPCC (see comments in Rodriguez-Bigas *et al*[23]). We therefore raise the question whether our mutation carriers would have been detected by applying the Bethesda criteria as a first preselection step prior to tumour tissue and mutation analysis. The evaluation of a patient for possible HNPCC by applying the Bethesda guidelines requires the patient's personal

history. When a patient does not meet the Bethesda criteria, his or her family history has to be considered, as in some cases the patient's history combined with the family history raises the suspicion of HNPCC. In fact, 19 of our 27 mutation carriers alone or in combination with their family history met the Bethesda criteria. Thus, these 19 patients could have been identified as possible HNPCC patients without the diagnosis of a skin tumour. However, in six mutation carriers (22%), neither their personal nor their family history pointed towards HNPCC according to the Bethesda definition. These six patients would not have been included in mutation analysis, and their relatives would not have been informed about their significantly higher tumour risk and would not have been offered regular HNPCC surveillance examinations or genetic testing.

Apart from these six patients without a personal or a family history of HNPCC, two further mutation carriers did not meet the Bethesda criteria, but their family history data were not available for testing of these criteria. This scenario, where the patient cannot be asked for family data, is indeed a frequent situation, for example, where the histopathologist makes the diagnosis of a sebaceous neoplasm and could therefore be the first person to raise the suspicion of HNPCC and initiate further evaluation (examination of MMR gene expression and/or microsatellite analysis). As others have pointed out previously, the rare sebaceous neoplasms should therefore be included in the catalogue of HNPCC specific malignancies listed in the Bethesda guidelines.[2 18] We feel confident that if MTS skin tumours were part of the Bethesda criteria, the suspicion of HNPCC could be raised earlier, in some cases even before an internal malignancy occurs.

In summary, we identified DNA MMR gene mutations in 66% of patients from a large MTS sample who had been pre-selected by tumour tissue analysis. As the vast majority of mutations were identified in *MSH2*, MTS shows a clear-cut genotype–phenotype correlation. This fact can be of benefit for mutation analysis in MTS patients or HNPCC index patients with reported MTS patients among their relatives. Interestingly, a remarkable proportion of the mutation carriers (more than 20%) would have been overlooked if the Bethesda criteria instead of the MTS phenotype had been applied for ascertainment.

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