Microsatellite Instability in Benign Skin Lesions in Hereditary Non-Polyposis Colorectal Cancer Syndrome

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The coexistence of cutaneous and extra-cutaneous malignancies within one family could be explained by shared genetic mechanisms such as common tumor suppressor gene mutations or oncogene activation, as well as mutations in DNA repair genes. Hereditary non-polyposis colorectal cancer syndrome (HNPCC) and its variant Muir–Torre syndrome (MTS) are caused by germline DNA mismatch repair gene mutations. Colonic and endometrial tumors from HNPCC patients exhibit microsatellite instability (MSI), as do sebaceous lesions in MTS. We recruited individuals from cancer prone families to determine if MSI is found in benign and malignant skin lesions and to assess whether MSI in the skin is predictive of genomic instability with susceptibility to tumors characteristic of HNPCC. One hundred and fifteen benign, dysplastic, and malignant skin lesions from 39 cancer prone families were analyzed. Thirteen benign skin lesions from three individuals belonging to two HNPCC pedigrees showed MSI. No mutations in hMSH2 and hMLH1 were found in two of the three individuals with RER+ skin lesions. We found MSI in non-sebaceous non-dysplastic skin lesions in HNPCC pedigrees. MSI was not found in skin lesions within other family cancer syndromes. These results have important clinical implications as the detection of MSI in prevalent readily accessible skin lesions could form the basis of noninvasive screening for HNPCC families. It may also be a valuable tool in the search for new mismatch repair genes. ***Key words: colon cancer/HNPCC/screening, skin. J Invest Dermatol 113:901–905, 1999***

Analysis of genetic changes in cutaneous lesions in family cancer syndromes such as familial melanoma and naevoid basal cell carcinoma syndrome has been useful in the identification of key tumor suppressor genes (Hussussain *et al*, 1994; Johnson *et al*, 1996). Common mechanisms of tumor induction are inactivation of tumor suppressor genes or activation of proto-oncogenes. More recently, the identification of microsatellite instability in tumors has led to the recognition that DNA mismatch repair genes are important in cancer development (Peltomaki *et al*, 1993).

DNA mismatch repair genes have been implicated in the pathogenesis of hereditary non-polyposis colorectal cancer syndrome (HNPCC) (Lynch *et al*, 1993). HNPCC predisposes to a variety of early onset neoplasms, most commonly carcinoma of the colon and endometrium. There is also an increased prevalence of malignancies of the stomach, small bowel, pancreas, ovary, ureter, and renal pelvis (Lynch *et al*, 1993; Watson and Lynch, 1993). HNPCC is a dominantly inherited condition thought to be responsible for approximately 0.5–13% of cases of colorectal cancer (Aaltonen *et al*, 1998). Muir–Torre syndrome (MTS) is characterized by sebaceous adenomas in addition to the spectrum of tumors usually seen in HNPCC pedigrees (Lynch *et al*, 1985). HNPCC families require regular screening with colonoscopy. Additional screening such as pelvic ultrasound, blood CA125 level, and upper gastrointestinal endoscopy is also important. Ascertainment of HNPCC families remains difficult as there are no clinical features that are pathognomonic of the condition (Aaltonen *et al*, 1998). At present the diagnosis of HNPCC in a family is made on the basis of the "Amsterdam criteria" (Vasen *et al*, 1991); the presence of at least three histologically verified cases of colorectal cancer in at least two generations, one being diagnosed before age 50 and one being a first degree relative of the other two, with familial adenomatous polyposis excluded. The Bethesda guidelines (Rodriguez-Bigas MA *et al*, 1997) have since been devised as a guide for which tumors should be tested for MSI. Screening for germline variants of the five DNA mismatch repair genes identified to date is time consuming and expensive, with a significant failure rate in mutation detection (Sheffield *et al*, 1993; Liu *et al*, 1996). The majority of HNPCC families have mutations in either hMLH1 or hMSH2 (Papadopoulos and Lindblom, 1997). Many families with a clear predisposition to both colonic and uterine tumors will, however, not have mutations and the next step is often to look for MSI in the tumors (Wijnen *et al*, 1998). This technique requires archival tumor material and the individual will already have had cancer by the time he or she undergoes screening. Recent publications have high-

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Abbreviations: HNPCC, hereditary nonpolyposis colorectal cancer syndrome; MSI, microsatellite instability; MTS, Muir–Torre syndrome; RER+, replication error phenotype.

lighted the need for appropriate strategies for HNPCC molecular screening, as it is highly likely that screening for cancer in association with prophylactic surgery can reduce cancer mortality in these families (Aaltonen *et al*, 1998; Wijnen *et al*, 1998). Clinical criteria exist for the definition of HNPCC (Vasen *et al*, 1991) but it is increasingly recognized that their validity is limited as they exclude many families with a clear inherited cancer predisposition likely to be caused by DNA mismatch repair gene mutations but who have extracolonic, rather than colonic tumors (Beck *et al*, 1997a).

Loss of DNA mismatch repair gene function results in a failure to repair incorrect replication of DNA. The consequent variation in length of microsatellite sequences seen in malignant HNPCC tumors is known as microsatellite instability (MSI), also termed the replication error (RER+) phenotype. In a large series of colorectal tumors from HNPCC kindreds, 92% showed a RER+ phenotype (Aaltonen *et al*, 1994; Liu *et al*, 1996). MSI is an unusual finding in benign and malignant skin tumors and has mainly been reported in sebaceous adenomas of individuals with Muir–Torre syndrome (Peris *et al*, 1997). In non-sebaceous skin lesions it has been demonstrated in actinic keratoses but again from one Muir-Torre kindred (Quinn *et al*, 1995) and in a minority of primary malignant melanomas (Walker *et al*, 1994; Peris *et al*, 1995; Tomlinson *et al*, 1996).

The aim of this study was to determine if microsatellite instability was present in benign, dysplastic, and malignant skin lesions in patients belonging to cancer prone families, including HNPCC pedigrees. The specific aim was to determine whether microsatellite instability in the skin may be predictive of genomic instability with susceptibility to internal malignancies.

MATERIALS AND METHODS

Recruitment and patient characteristics We recruited patients from the Family Cancer Clinic in the Dermatology department at the Royal London Hospital and the Family Cancer Clinic at St Mark's Hospital, Northwick Park. All patients included in this study were referred for benign, dysplastic, or malignant cutaneous lesions with a pedigree strongly suggestive of a family cancer predisposition. Most pedigrees included a variety of internal tumors as well as melanoma and/or nonmelanoma skin cancers. Three patients referred for benign and dysplastic skin lesions belonged to HNPCC pedigrees according to the Amsterdam criteria. Fifteen families had a history of melanoma (at least two melanomas within the pedigree) with multiple atypical naevi, but 13 of these 15 families were characterized by additional clustering of multiple internal tumors. No Gorlin's families were included.

Data and sample collection Data on family history and full skin phenotype was collected. Blood samples and biopsy of benign or malignant skin lesions were taken during their visit to the clinic. Cutaneous lesions sampled included 44 melanocytic naevi, eight seborrhoeic warts, eight solar keratoses, three benign lentigines, four Bowen's disease, one sebaceous epitheliomas, four squamous cell papillomas, three sebaceous adenomas, 13 melanomas, 21 basal cell carcinomas, and six squamous cell carcinomas.

DNA extraction and analysis These 115 skin lesions were screened using a range of microsatellite markers (see **Table I**). All primer pairs used (Research Genetics, Huntsville, AL) had a reported heterozygosity score of greater than 80%. Where few markers are listed, there was either limited DNA because of a small original sample, or difficulty in amplifying the extracted DNA, usually from archival samples. DNA was obtained from paraffin-fixed material using microdissection of unstained sections to enrich for tumor tissue. Microdissection was performed by a dermatologist trained in dermatopathology (VJS) and original pathologic reports were reviewed. In cases of uncertainty the slides were reviewed by an independent dermatopathologist. Once the microsatellite analysis was completed the samples showing MSI were reviewed by a dermatopathologist who was blinded to the original reports. Archival tissue DNA was extracted by incubation at 56°C in Chelex 10% and proteinase K 0.2 mg per ml, followed by boiling for 8 min to inactivate the enzyme. DNA was extracted from blood and fresh tissue using standard techniques.

PCR was carried using a Robocycler Gradient 96 thermal cycler (Stratagene, La Jolla, CA), using primers (Research Genetics) end-labeled with $^{33}\gamma$ ATP (Amersham, Little Chalfont, Bucks, UK). PCR products were resolved on 5% polyacrylamide gels and visualized on a Storm 840 Molecular Dynamics Imager.

The PCR protocol was as follows: 3 min at 95°C, 1 min at 55°C, and 1 min at 72°C followed by 29 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C followed by 1 cycle of at 95°C, 1 min at 55°C, and 10 min at 72°C. Microsatellite instability was defined by the presence of additional novel bands in DNA from lesional skin compared with DNA from normal skin or blood control. There are, as yet, no internationally agreed criteria for the detection of the MSI phenotype in extra-colonic tissue, although criteria have been developed for classification of microsatellite instability in the colon (Boland *et al*, 1998).

Detection of mutations in peripheral blood was carried out on subjects with MSI in the skin using single strand conformational polymorphism (SSCP) and direct DNA sequencing (Beck *et al*, 1997b) of the exons of hMSH2 and hMLH1.

RESULTS

Thirteen benign skin lesions from three individuals belonging to two HNPCC families showed MSI using a range of microsatellite markers. **Table I** shows the frequency of MSI detection in a variety

Table I. Microsatellite instability (MSI) in benign cutaneous lesions in cases 1 and 2[a]

	9S171	17S785	17S796	9S162	13S155	9S176	17S579	1S214	10S185	1S201	9S160	No. MSI/ amplified
Case 1												
sebaceous adenoma 36	●	●	●	●	●	●	NT	NT	NT	NT	NT	6/6
sebaceous adenoma 37	●	●	●	●	NP	●	NT	NT	NT	NT	NT	5/5
seborrhoeic wart 38	●	●	●	●		●	NT	NT	NT	NT	NT	5/6
seborrhoeic wart 39	●	●	●	●	●	NP	NT	NT	NT	NT	NT	5/5
seborrhoeic wart 40			●	NP			NT	NT	NT	NT	NT	1/5
inflamed early seborrhoeic wart 41	●	●	NP	NP	NT	●	NT	NT	NT	NT	NT	3/3
seborrhoeic wart 100	NT	NP		NP	NP	NP	●	NP	NT	NP	NT	1/2
sebaceous tumour 102	NP	NP	NP	NP	NP	NP	●	NP	NT	●	NT	2/2
sebaceous epithelioma 186	nnNP	NT	●	NT		●	NT			NT	●	3/6
sebaceous epithelioma 187	●	NT	●	NT	NP	●	NT	●	●	NT	●	6/6
Case 2												
seborrhoeic wart 42		●	●	●	●		NT	NT	NT	NT	NT	4/6
stucco keratosis 43	●	●	●	NP	●		NT	NT	NT	NT	NT	4/5
inflamed early seborrhoeic wart 44	●	●	NP	NP	●		NT	NT	NT	NT	NT	3/4
seborrhoeic wart 45		●	NP	●	●	●	NT	NT	NT	NT	NT	4/5

[a]●, MSI; NP, no product; NT, not tested; all markers demonstrated MSI of at least one lesion

of skin lesions from case 1 and case 2. In case 1, two sebaceous adenomas, five seborrhoeic warts, two sebaceous epitheliomata, and one sebaceous tumor were biopsied for DNA extraction. In case 2, no sebaceous adenomas were found and three seborrhoeic warts and one stucco keratosis were sampled for genetic analyses. In case 3, two stucco keratoses were sampled and screened with 10 markers. One squamous cell papilloma of case 3 showed MSI at one marker but failed to amplify for most of the other markers studied.

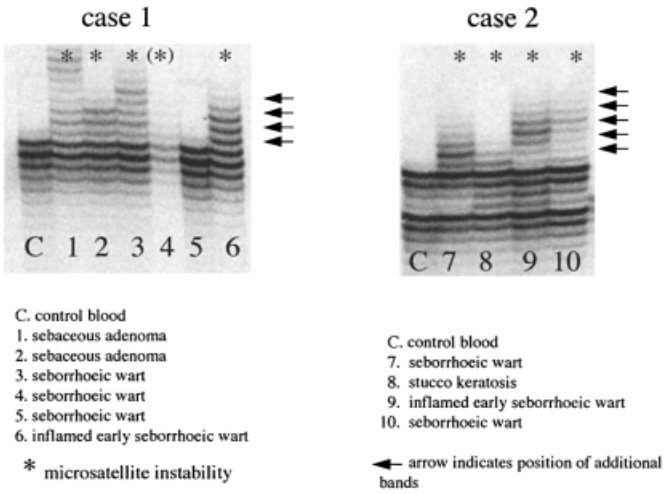

Figure 1. Polyacrylamide gel electrophoresis showing microsatellite instability at D17S785 in benign skin lesions of cases 1 and 2, respectively.

This may be due to the fact that the sampled skin lesions were very small. **Figure 1** shows MSI in several skin lesions in case 1 and case 2, respectively. The three individuals with RER+ skin lesions were from families which fulfilled the Amsterdam criteria for HNPCC. Only one of these two families, however, had already been characterized as an HNPCC pedigree prior to attending the dermatology clinic. The other case was only recognized as belonging to an HNPCC/MTS pedigree after taking a full family history in the clinic. The presence of sebaceous adenomas confirmed that this was a Muir-Torre kindred. All skin lesions biopsied from these two probands were RER+. No malignant cutaneous or extracutaneous lesions were available from these two cases for analyses of genetic alterations. The personal and family history of case 1 and 2 was as follows: Case 1 had a personal history of breast cancer and sebaceous adenomata and epitheliomata, and a family history of colon, bladder, stomach, kidney, and ovarian cancers in first and second degree relatives (**Fig 2**). Case 2 had a personal history of cancers of the colon and uterus, a first degree relative with colon cancer and five second degree relatives with malignancies, including colon, cervix, breast, uterus, and larynx (**Fig 3**). One of her maternal aunts had three primary tumors (uterus, colon, and breast). Case 3, a maternal cousin of case 2, showed MSI in one benign skin lesion. No mismatch repair gene mutation was detected in lymphocyte DNA from case 1 and 2 by SSCP or direct sequencing of all exons.

Of the remaining 102 benign or malignant skin lesions from 40 individuals from 37 cancer prone families, none were found to be RER+. Histologic subtypes of skin lesions screened with a range of microsatellite markers from non-HNPCC pedigrees is shown in Table II. None of these families fitted the Amsterdam criteria for HNPCC. Most of these families were characterized by cancer predisposition to a variety of internal malignancies with or without

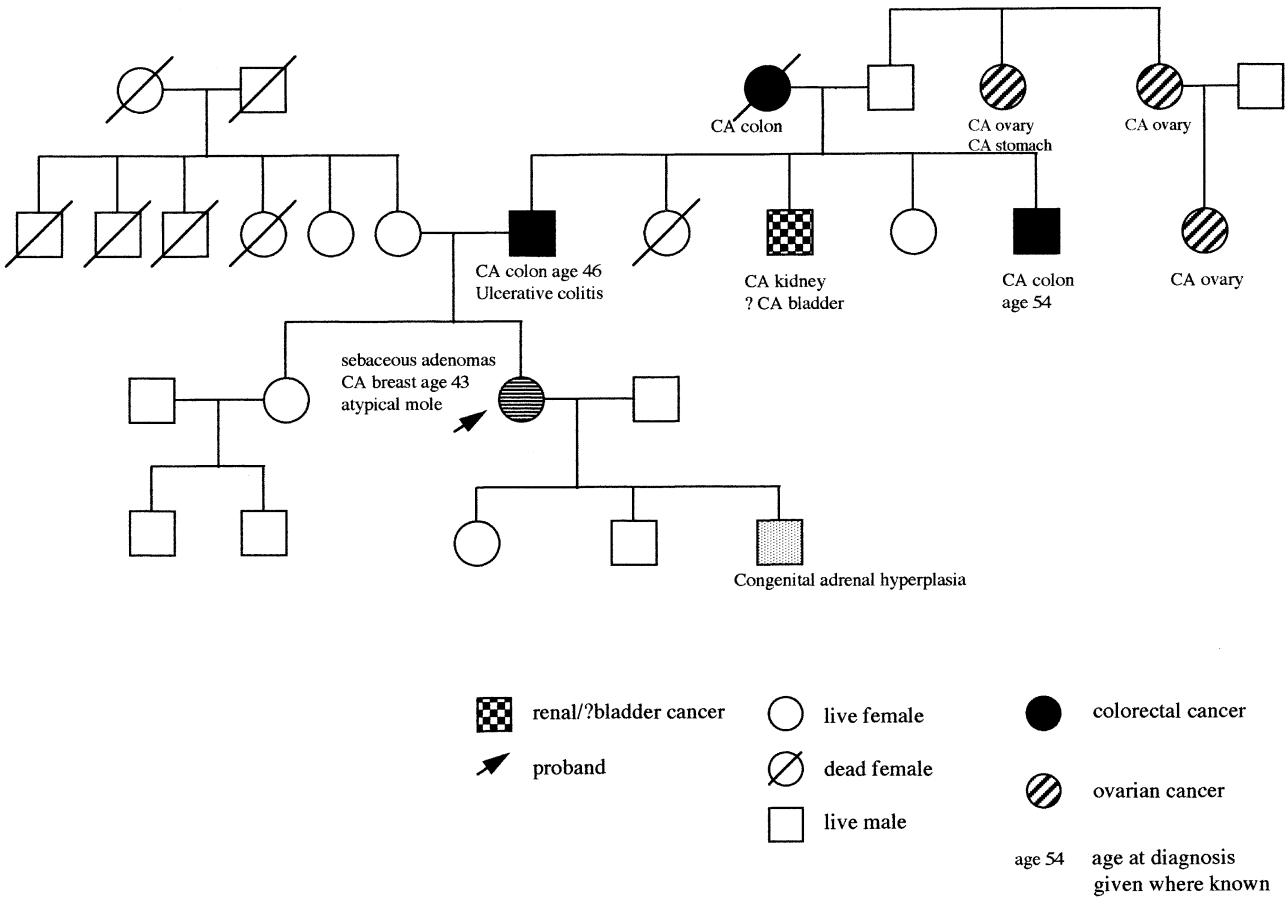

Figure 2. Family tree of case 1. →, Proband.

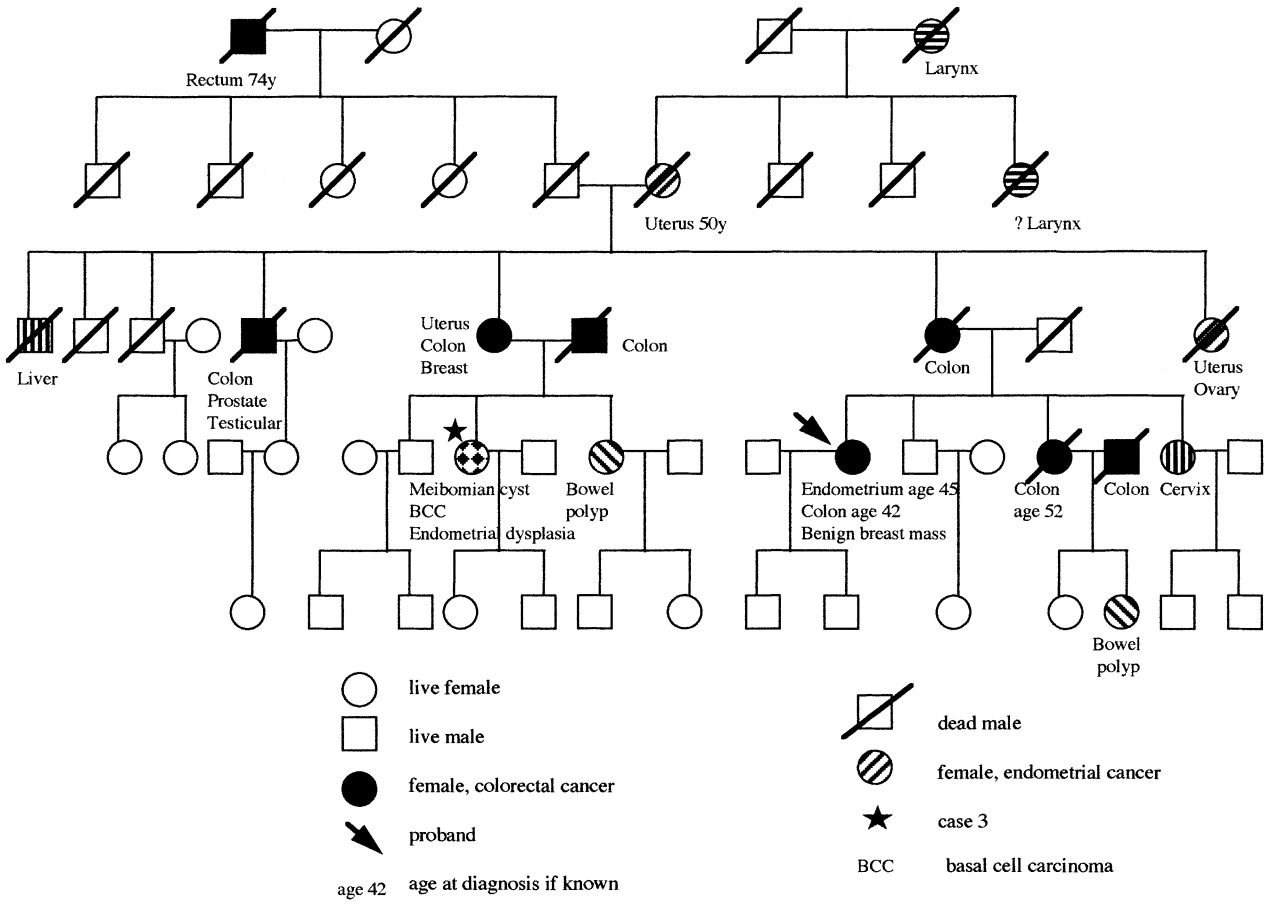

Figure 3. Family tree of case 2 and case 3. →, Proband; *maternal cousin.

Table II. Histologic subtypes of skin lesions from non-HNPCC family members and microsatellite status

Histologic diagnosis	No. MSI+	No. MSI–	Total no. of lesions
Melanocytic naevus	–	44	44
Malignant melanoma	–	13	13
Basal cell carcinoma	–	21	21
Seborrhoeic wart	–	2	2
Actinic keratosis	–	8	8
Lentigo	–	3	3
Bowen's disease	–	4	4
Sebaceous epithelioma	–	–	–
Squamous cell papilloma	–	1	1
Sebaceous adenoma	–	–	–
Squamous cell carcinoma	–	6	6
Total	0	102	102

cutaneous malignancies. Of the family cancer syndromes characterized by a clustering of internal and cutaneous malignancies, melanoma was found in 17 families, basal cell carcinoma in six families, and both in four families.

DISCUSSION

We have detected microsatellite instability in nondysplastic, nonsebaceous skin lesions in three affected individuals from HNPCC families. This RER+ phenotype was not associated with mutations in hLMH1 or hMSH2. Case 1 had not previously been identified as belonging to a HNPCC/MTS family despite her strong family history of cancer and her family was not part of a screening programme at the time of presentation. Case 2 had a personal history of uterine and colon primaries whilst case 3 (maternal cousin of case 2) had basal cell carcinoma but no internal tumors. She underwent numerous uterine biopsies that revealed cytologic atypia but no frank malignancy.

Investigation of genetic alterations in the skin of family cancer patients is a powerful tool as cutaneous lesions are easily identified, readily accessible, and often numerous. PCR-based microsatellite analysis allows for the detection of both genomic instability and nonrandom chromosome loss as multiple lesions can often be sampled.

The detection of MSI in seborrhoeic warts and stucco keratoses indicates that the genomic instability characteristic of malignant tumors from HNPCC families may also be present in benign skin lesions. Indeed, this may even be detected before the onset of malignancies: case 3 has not developed any internal malignancies but showed microsatellite instability in one benign skin lesion. Common cutaneous lesions are more readily accessible for DNA analysis than internal neoplasms such as malignancies of the colon and uterus. Although DNA extraction from some of the older paraffin blocks may have been unreliable, assessment of MSI in DNA from small frozen skin samples was straightforward. As benign skin lesions may in the future be used for the sole purpose of MSI detection in these families, the problem of extraction from paraffin embedded tissue encountered for some of the lesions screened in this study will no longer be an issue. MSI was not detected in any of 102 benign or malignant skin lesions from non-HNPCC family cancer patients, strongly suggesting that the RER+ phenotype in the skin is specific to HNPCC/MTS families. Furthermore, these results confirm that MSI is a very rare event in

melanocytic naevi, melanomas, and basal cell carcinomas within non-HNPCC pedigrees. Microsatellite instability was not detected in a previous population-based study of 27 seborrheic warts, which supports the predictive value of a positive RER phenotype in seborrhoeic warts within HNPCC pedigrees (Charman *et al*, 1998). Benign lesions such as seborrhoeic warts are prevalent enough in the normal population to be useful for cancer screening.

A recent survey found that seborrhoeic warts were present in 33% of 100 general medical inpatients.[2] Data from the U.K. and Australia have shown that these lesions, although more prevalent in older age groups, are also found commonly in subjects aged 50 or less.[2] Although it has been observed that seborrhoeic warts may be more common in cancer prone individuals this association has been controversial (Grob *et al*, 1991; Yamamoto and Yokoyama, 1996). Dantzig (1973) was the first to report the appearance of multiple seborrhoeic warts in individuals with cancer and further cases reported were mainly associated with gastrointestinal tumors. Seborrhoeic warts tend to appear from the third decade and can be transmitted as an autosomal dominant trait (Lynch *et al*, 1982; Yamamoto *et al*, 1996). What causes the induction of these lesions is unclear but both genetic factors and sun exposure have been implicated (Yeatman *et al*, 1997).

Our data suggest that detection of MSI in common benign skin lesions of patients belonging to HNPCC families may have important clinical implications, as detecting MSI in the skin could potentially be used in clinical practice to screen individuals in families at risk. At present, detection of DNA mismatch repair gene mutations is relatively expensive and laborious with incomplete predictive value.

The detection of MSI in colon tumors requires archival paraffin embedded tissue with potential problems in accessing samples and DNA amplification. Skin lesions will also be invaluable for the detection of MSI in family members who have not yet developed cancer and may be potentially affected. In conjunction with a family history of multiple cancers suggestive of HNPCC/MTS, detection of microsatellite instability in the skin may be used to direct appropriate cancer screening and may be additionally useful in the future for the identification of new mismatch repair genes. Further studies collecting a wide range of HNPCC families will determine the specificity and sensitivity of this screening method.

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