Studies of Hereditary Nonpolyposis Colorectal Cancer in Japan

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INTRODUCTION

Epidemiologic data have suggested that the development of cancer is largely influenced by environmental factors. Recent studies, however, have showed that inheritance has a more significant part in carcinogenesis and in the management of cancer than previously believed. Colorectal cancer has a major role in the prologue of revolutionary developments in cancer research and control in the 21st century. In 1973, Knudson[1] proposed the existence of 2 clusters of all site-specific cancers; genetic or prezygotic, and epigenetic or postzygotic. He then provided us a useful classification of cancer named "Oncodeme," based on the environmental and genetic relationship in oncogenesis, for the construction of a cancer control strategy (Table 1). Therefore, it is important to identify the environmental and genetic influences of a patient with cancer to prolong survival. Clinical observations of patients with remarkable aggregation of colorectal cancer and, or associated with, specific clinical markers, showed several types of hereditary colorectal cancer; divided roughly into the polyposis syndrome, and hereditary colorectal cancer not associated with polyposis[2,3] (Table 2).

In 1913, the year immediately before the report of successful production of the rabbit tar cancer by K. Yamagiwa, professor of pathology of the University of Tokyo, Aldred Warthin, a pathologist at the University of Michigan School of Medicine, first reported on "cancer families."[4] He studied 4 families with a remarkable conjunction of multiple cancers that were chosen from 1600 histologically proven records of patients with carcinoma. One of these families, "family G," was later studied in 1971 by Lynch and Krush, who proposed a clinical category termed "cancer family syndrome."[5] In 1981, one of the author's of this article, Utsunomiya, used the term "nonpolyposis familial large bowel cancer" to include the site-specific familial large bowel cancer and the cancer family syndrome.[2] In 1985, Lynch et al.[6] defined Lynch syndrome I and II, shown in Table 2. The term hereditary nonpolyposis colorectal cancer (HNPCC) comprises these 2 syndromes.[6]

In 1993, defective mismatch repair genes in germlines were found to be responsible for HNPCC, as discussed in the next section. The discovery is regarded as a particularly outstanding event in cancer research that has happened to elucidate one of the major mechanisms of oncogenesis. These genes are a second class of susceptibility genes after tumor suppressor genes, the first class genes represented by the *APC* gene in familial adenomatous polyposis (FAP). Knowledge of these genes raises the possibility of cancer predisposition testing for primary and secondary prevention. How to apply those basic discoveries efficiently, as well as harmlessly, to the reduction of disease morbidity and mortality—so called translational research,[7] is now urgently needed.

CLINICAL AND EPIDEMIOLOGIC ASPECTS

History of the Studies on Hereditary Nonpolyposis Colorectal Cancer (HNPCC) in Japan

Case reports

One of the earliest case reports in Japan on a familial occurrence of colorectal cancer not associated with polyposis was made by Kameya et al. in 1969.[8] One of the author's of this current article, Utsunomiya, studied the same family in 1981 and identified 5 family members with colorectal cancer at the average age of 41.7 years. Many other family members were affected with diverse types of cancers; such as cancer of the stomach, duodenum, lung, and lymph nodes[9] (Fig. 1). One of the patient's (case 1) had multiple polypoid lesions in the ascending colon, which included 3 small advanced cancers and several flatt or pedunculated displastic adenomas (Fig. 2). This was followed by a series of similar case reports, that totaled 49 families (219 cases) in early 1990.[10,11] At present, the largest pedigree is the

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Table 1. Classification of cancer based on environmental and genetic relationship in carcinogenesis.

	Etiologic events		Other terminology		Proportion
	Environmental	Genetic			
Oncodeme 1	average	average	random cancer	unavoidable cancer	? 20%
Oncodeme 2	very increased	average	environmental cancer	occupational cancer virus origin	
Oncodeme 3	moderately increased	moderately increased	multifactorial cancer	familial cancer	? %
Oncodeme 4	average	very increased	hereditary (single gene cancer)	familial cancer	1% to 5%

Modified from Oncodeme by Knudson.[1]

Table 2. Classification of hereditary colorectal cancer.

Clinical symptom	Histology of primary lesion	Heredity	Genotype	
			Gene	Locus
A. Polyposis syndrome				
Familial adenomatous polyposis (FAP)	adenoma	AD	*APC*	5q21-22
Gardner syndrome	adenoma	AD	*APC*	5q21-22
Turcot syndrome Recessive form	adenoma	AR	*MRG*	
Dominant form	adenoma	AD	*MRG* or *APC*	5q21-22
Attenuated FAP	adenoma	AD	*APC*	5q21-22
Peutz-Jeghers syndrome	hamartoma	AD	*STK11*	19p13.3
Juvenile polyposis	hamartoma	AD	*PTEN*	10q23.3
Cowden disease	hamartoma	AD	*PTEN*	10q23.3
B. Hereditary nonpolyposis colorectal cancer (HNPCC)				
Lynch syndrome I (familial site specific colorectal cancer)		AD	*MRG*	
Lynch syndrome II (cancer family syndrome)		AD	*MRG*	
Muir-Torre syndrome		AD	*APC*	
Hereditary solitary polyps (Woolf)	adenoma	AD	*APC*	5q21-22
Hereditary flat adenoma syndrome (Lynch)	adenoma	AD	*APC* *MRG*	5q21-22

AD, autosomal dominant; AR, autosomal recessive; MRG, mismatch repair gene.

one reported by Shozo Baba and colleagues, who identified 24 patients with colorectal cancer among 148 family members of 5 generations (Shozo Baba, written communication, August 1996).

Nationwide survey

The initial nationwide survey on familial colorectal cancer, performed by Utsunomiya in 1972, collected information on 37 families with HNPCC, in addition to many polyposis and related conditions.[12] To continue this study and to preserve the information, the Polyposis Center was established in 1976.[12–14] At the end of 1997, the center's registration database contained 123 families with HNPCC, in addition to 792 with familial adenomatous polyposis, 180 with Peutz-Jeghers syndrome, and 12 with juvenile polyposis (Takeo Iwama, written communication, August 1996). The first nationwide survey restricted to HNPCC was conducted by questionnaires to 60 member institutions, as an activity of the Japan Research Society for Colorectal Cancer (JRSCC) in 1991 by Komi and Kunitomo of the Tokushima University, with the collaboration of Utsunomiya.[15] At the second survey done at the 43rd annual meeting of the JRSCC in 1995, chaired by Shozo Baba in Hamamatsu, Japan, 109 institutions responded to a questionnaire for study purposes.[16]

Meetings

In 1989, the 4th International Symposium subtitled as the Hereditary CRC (colorectal cancer) was organized by Utsunomiya and Lynch in Kobe, Japan. Multidisciplinary facets of the studies on polyposis and nonpolyposis cancer were discussed with a gathering from 18 countries, and a series of collaborative study activities ensued. The nomenclature of HNPCC was internationally approved. The International Collabora-

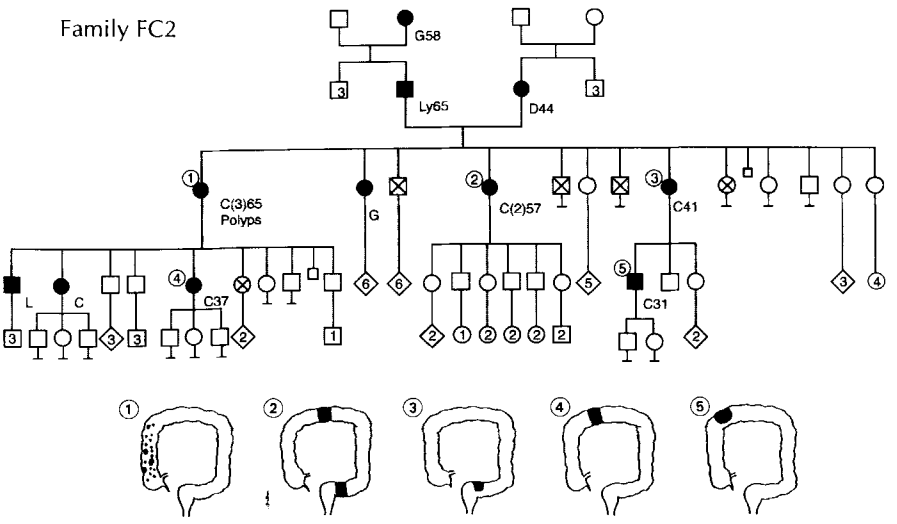

Fig. 1. The pedigree of the first family reported in Japan to have colorectal cancer not associated with polyposis (from Kameya et al.[8] and Utsunomiya et al.[9]). Squares, men; circles, women; open symbols, no tumor; solid symbols, hereditary nonpolyposis colorectal cancer (HNPCC) patient with cancer; cross oblique bars, death with disease other than cancer.

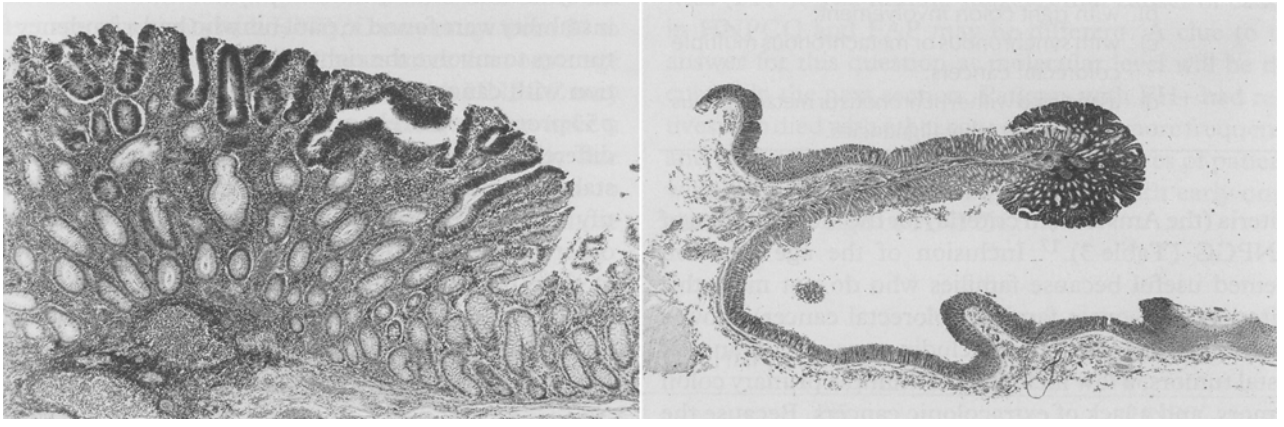

Fig. 2. Microscopic finding of 2 adenomatous lesions in the ascending colon of patient 1 of FC 2 (provided by S. Kameya). (**A**) a flatt adenoma (hematoxylin and eosin stain; original magnification, × 7); and (**B**) a pedunculated adenoma.

tive Group on HNPCC (ICG-HNPCC) was initiated immediately after the last session.[17] In 1993, the 48th Congress of the Japanese Society of Coloproctology, presided over by Utsunomiya, was held as a part of the first Japanese Digestive Disease Week. A satellite symposium entitled as "New Insights into Molecular Biology of CRC" was timely, with important panelists such as Lynch, de la Chapelle, and Nakamura, who showed that our astute observations on rare cases of hereditary tumors, combined with the current knowledge on molecular biology, could provide us with an invaluable opportunity for understanding the development of cancer. In 1997, a symposium, Familial Cancer and Prevention, was organized by Utsunomiya as a function of one of the projects chaired by Weber within the UICC Cancer Prevention and Epidemiology Program chaired by Tominaga. At the symposium, diverse facets of translational research and ethical, legal, and psychological issues were discussed with 343 attendants from 19 countries. HNPCC and related topics were prominent in 26.7% of the 215 papers.[18]

Diagnostic Criteria

Establishing the diagnosis of HNPCC is especially difficult, because of the lack of a pathognomic phenotypic marker. Diagnosis is currently based upon the combined patient and family data. During the last decade, diverse diagnostic criteria have been proposed (Table 3).

The Amsterdam minimum criteria

To provide a basis for uniformity in (multicenter) studies, in 1991 the ICG-HNPCC proposed the minimum

Table 3. Diagnostic criteria of hereditary nonpolyposis colorectal cancer (HNPCC).

Lynch criteria for Cancer Family Syndrome
1. An increased incidence of adenocarcinoma, primarily in the colon and endometrium
2. An increased incidence of multiple primary malignant neoplasm
3. Early stage onset of an autosomal dominant inheritance

Amsterdam minimum criteria for HNPCC
1. Three or more relatives with histologically verified colorectal cancer, 1 of whom is a first-degree relative of the other 2
2. Colorectal cancer affecting at least 2 generations
3. One or more colorectal cancer cases diagnosed before age 50

Japanese clinical criteria for HNPCC
1. A case with 3 or more colorectal cancers within the first-degree relatives
2. A case with 2 or more colorectal cancers within the first-degree relatives meeting the following criteria:
   a) age at onset of colorectal cancer(s) being earlier than 50 years old;
   b) with right colon involvement;
   c) with synchronous or metachronous multiple colorectal cancers;
   d) associated with synchronous or metachronous extracolorectal malignancies.

criteria (the Amsterdam criteria) for the identification of HNPCC (Table 3).[17] Inclusion of the age criterion seemed useful because families who do not meet this criterion (late-onset familial colorectal cancer) show a distinct clinical phenotype including a preponderance of distal tumors, a low incidence of multiple primary colon tumors, and a lack of extracolonic cancers. Because the clinical features of such families resemble those of sporadic (common) colorectal cancer, it is conceivable that late-onset familial colorectal cancer simply represents familial clustering of colorectal cancer by chance or as a result of shared environmental influences. A recognized disadvantage of the Amsterdam criteria is that extracolonic tumors associated with HNPCC are not included. This may lead to a delay of diagnosis of HNPCC in families with such tumors. Therefore, it must be emphasized that families, who do not meet all Amsterdam criteria, may also represent HNPCC and may need surveillance.[19]

The Japanese clinical criteria

A set of clinical criteria for HNPCC was proposed by the JRSCC for use in the screening of possible patients with HNPCC at the 34th Meeting (March 1991, Tokushima, Japan) (Table 3) and published at the 5th International Symposium on CRC in Turin, Italy, 1991.[15] These criteria were intended to identify as many patients with HNPCC as possible and to overcome the difficulty or impossibility of obtaining a complete family history (for example, relatives may not exist or may die before the occurrence of colorectal cancer). Elimination of histologic verification from the criteria and setting of subcriteria in high-risk patients (including age at onset, tumor location, multiplicity of colorectal cancer, and complication by extracolorectal malignancy), together with relaxation of the family history requirement, are peculiar to these criteria. While the Amsterdam criteria is a precise tool for understanding molecular genetic pathogenesis of HNPCC, the latter criteria have been found to be very useful for early identification of persons at high risk for colorectal cancer.

Diagnostic criteria and microsatellite instability

Microsatellite instability in somatic cells is a phenotype of HNPCC (see the section on molecular aspects). According to Muta et al.,[20] the patients with microsatellite instability accounted for 62% of group A of the Japanese clinical criteria (a confirmed group) and 35% of group B (a high-risk group). Conversely, only 5% of the randomly selected colorectal cancer group had microsatellite instability. Furthermore, tumors positive for microsatellite instability were found in patients who had a tendency for tumors to involve the right side of the colon, an association with cancers in other organs, a lower incidence of p53 protein positively, and a higher proportion of poorly differentiated cancers. The presence of microsatellite instability, in concert with the clinical criteria, may identify legitimate cases of HNPCC in patients that might otherwise be excluded by the minimum criteria. Sasaki et al.,[21] however, found no germline mutations of the *hMLH1* gene and detected only 2 somatic missense mutations among the 43 tumors examined from 23 Japanese patients with multiple primary cancers.

Proportion of HNPCC in General Colorectal Cancer

The incidence of patients with family history of colorectal cancer not associated with familial adenomatous polyposis (FH+) was 6.5% in 15,369 patient records preserved in the JRSCC registry.[22] Among the 32,470 cases of colorectal cancer in patients, accumulated from the first HNPCC national survey, a positive family history was found in 1384 cases (4.2%); of those, 777 families (2.4%) were categorized to be HNPCC according to the Japanese clinical criteria, and 69 families (0.2%) were so categorized according to the Amsterdam minimum criteria.[16] At the second survey, 73,785 patients with colorectal cancer were identified; of those, 4109 patients (1740 families) or 2.4% were categorized to have HNPCC as determined by the Japanese clinical criteria. There were 1366 patients (410 families) who fit criterion A, 1149 (543) who fit criterion B-a, 1402 (684) who fit criterion B-c, and 534 (256) who fit criterion B-d.[12] Whereas there were 394 patients (109 families) or 0.15% who met the Amsterdam minimum criteria.[16]

The purely coincidental association of colorectal cancer in one of the parents was estimated to be 3.2%, considering that the prevalence of colorectal cancer in the general population in Japan was 1.6% in 1975.[23] Therefore, the relative proportion of genetically determined familial colorectal cancer would be approximately 1.0% to 3.2%.

Every dominantly inherited condition shows nonfamilial, or single case, disease in a certain proportion mainly due to de novo mutation, and possibly due to incomplete penetrance, limited assessment, phenocopy, etc. This proportion is larger in the conditions with the early onset phenotype than in those with the late onset, because the fresh mutation rate is related to the natural selection rate—or adversely related to relative fitness (representing reproductive ability). The proportion for the single case disease is 49.6% in familial adenomatous polyposis that has an average age at diagnosis of 37.5 years of age for men and of 30.4 years of age for women, while the proportion for the single case disease is much larger, at 66.7%, in Peutz-Jeghers syndrome, that has a much younger average age of diagnosis at 23.0 and 26.0 years of age, respectively.[14] We could refer to the above data for estimation of the nonfamilial proportion of HNPCC. Since the onset age of patients with HNPCC may range from 30 to 60 years of age, that is 10 or 20 years more than onset age for the other 2 conditions, the patient with HNPCC may survive for a longer reproductive period, and so have a smaller proportion of the single case. On family size distribution, however, HNPCC shows a similar pattern to familial adenomatous polyposis (Table 4).[9,14] From those results, it was assumed that the nonfamilial case could occupy 30% to 40% of the HNPCC population, and the proportion of HNPCC could possibly account for 1.3% to 5.3% of the Japanese population with colorectal cancer.

Characterization of HNPCC

Hereditary versus sporadic colorectal cancer and familial adenomatous polyposis

A study on FAP was made by Utsunomiya et al. in 1981 to characterize HNPCC by using the informative materials registered in the Polyposis Center and by extensively analyzing the personal background information.[9,14] The series of HNPCC patients with a family history positive for colorectal cancer (FH+), corresponded fairly well with those selected by the Japanese criteria. In patients with FH+, the lesions were more frequently multiple, shifted to the right colon, and appeared in patients at much younger ages than in patients with sporadic colorectal cancer (FH–) (Table 5). In patients with FAP-associated cancer, cancers occurred in patients at even younger ages, and the lesions were more frequently multiple than in patients with FH+, but they were distributed in a manner similar to that of lesions in patients with (FH–). The finding suggests that causes of cancer in HNPCC and FAP may be different. A clue to the answer for this question at molecular level will be discussed in the next section. Patients with FH+ had relatives who died with other cancers 3 times more frequently and at much younger ages than did relatives of patients with FH– (Table 6). Having a parent with early onset cancer is one sign of HNPCC.

Table 4. Characteristics of colorectal cancer according to FAP, PJS, or HNPCC.

Variable	FAP			PJS			HNPCC	
Average age at onset (y)								
Women	30.4			26.0			45 (19 to 69 y)	
Men	37.5			23.0				
Total no. with cancer	250			90			estimated > 39	
Nonfamilial cases (%)	124	49.6%		60	66.7%		estimated 30% to 40%	
Total familial cases	126	50.4%	100.0%	30	33.3%	100.0%	39	100.0%
2 affected	50	20.0%	39.7%	18	20.0%	60.0%	21	53.8%
3 affected	32	12.8%	25.4%	4	4.4%	13.3%	8	20.5%
4 affected	14	5.6%	11.1%	2	2.2%	6.7%	3	7.7%
5 affected	8	3.2%	6.3%	4	4.4%	13.3%	4	10.3%
6 affected	9	3.6%	7.1%	0	0%	0%	2	5.1%
7 affected	3	1.2%	2.3%	0	0%	0%	1	2.6%
8 affected	4	1.6%	3.2%	0	0%	0%	0	0%
9 affected	4	1.6%	3.2%	1	1.1%	3.3%	0	0%
10 affected	1	0.4%	0.8%	0	0%	0%	0	0%
11 affected	1	0.4%	0.8%	1	1.1%	3.3%	0	0%

FAP, familial adenomatous polyposis; PJS, Peutz-Jeghers syndrome; HNPCC, hereditary nonpolyposis colorectal cancer. Modified from Utsunomiya et al.[9,14]

Table 5. Comparison of FAP cancer and HNPCC.

	Nonpolyposis group		
	Family history negative (Sporadic CRC) (n = 241)	Family history positive (HNPCC) (n = 60)	FAP group (n = 229)
Average age	57.8 y	47.7 y	39.0 y
Right cancer	23.0%	39.0%	25.0%
Multiple cancer	3.0%	26.7%	37.6%

FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer; CRC, colorectal cancer. Modified from Utsunomiya et al.[9]

Table 6. Association of extracolonic cancer in family members of nonpolyposis colorectal cancer patients with positive and negative colorectal cancer family history.

Cancer in family member	Colorectal cancer patients	
	Family history negative (Sporadic CRC) (n = 122)	Family history positive (HNPCC) (n = 48)
Patient with other cancer	35	43
Number per family	0.28	0.90
Stomach	10 (28.6%)	17 (39.6%)
Uterine (origin unknown)	2 (5.7%)	5 (11.7%)
Endometrium	0	1 (2.3%)
Breast	0	4 (9.3%)
Other organs	23 (65.7%)	16 (37.2%)
Average age of death (y)	66.7	47.7

CRC, colorectal cancer; HNPCC, hereditary nonpolyposis colorectal cancer. Modified from Utsunomiya et al.[9]

Tumor spectrum

As seen in Table 7, the rate of gastric cancer in the extracolonic tumors of the Japanese series was apparently increased in comparison to the data from Western countries. In the earlier generation of the family G pedigree, reported by Warthin in 1913 and 1925, in the original family of Lynch syndrome II, an excess of gastric cancer was seen instead of colorectal cancer and endometrial cancer, but this incidence declined in the later generation.[6] The trend has been seen in the United States, where there has been a marked decline in incidence of gastric cancer and increase in colorectal cancer.

Table 7. Tumor spectrum in hereditary nonpolyposis colorectal cancer (HNPCC) pedigree in various countries.

Variable	Country, Reporter				
	Japan National survey series 1991	Japan Authors' series	Finland Mecklin[24] 1991	USA Lynch[25] 1988	The Netherlands Vasen[26] 1986
No. of pedigrees	777	39		10	22
Extracolonic tumor	152 100.0%	15 100.0%	172 100.0%	151 100.0%	55 100.0%
Stomach	69 45.4%	6 40.0%	29 16.8%	13 8.6%	5 9.1%
Uterus[a]	9 5.9%	2 13.3%			
Endometrial	15 9.9%	0 0.0%	40 23.3%	45[b] 29.8%	14 25.5%
Breast	9 5.9%	3 20.0%	8 4.7%	17 11.3%	5 9.1%
Urogenital	14 9.2%	0 0.0%	10 5.8%	17 11.3%	7 12.7%
Lung	7 4.6%	1 6.7%	4 2.3%	1 0.6%	1 1.8%
Others	29 19.1%	3 20.0%	81 47.1%	58 38.4%	23 41.8%

[a]Uterine cancer, origin unknown; [b]female genital organ. Modified from Utsunomiya et al.[9]

These findings suggest that HNPCC gene(s) could manifest gastric cancer in an environment favorable for gastric carcinogenesis.

There were 11 pedigrees reported as "familial gastric cancer" in Japanese literature that had 3 or more close relatives with gastric cancer in a family. Five pedigrees were found to contain relatives with the extragastric cancers.[27] A nationwide inquiry survey on gastric cancer, performed by Kino in 1995 as an activity of the JRSCC, has accumulated information from 488 patients with gastric cancer who were known to have 2 or more family members with gastric cancer in second- or first-degree relatives. The incidences were 1% to 4% in the series of gastric cancer that responded from 33 collaborating institutions. Thirty-six or 7.4% of proband patients had extragastric cancer, which includes 16 patients with colorectal cancer and 11 with cancer of the uterus. Only 1 family fit the Amsterdam criteria.[28] When all of the families with relatives who were given a diagnosis of colorectal cancer or other cancerous tumors were identified, 10% to 20% of those with familial gastric cancer fit the clinical category for HNPCC.

Akiyama et al.[29] studied familial gastric cancer kindred who were selected according to the gastric version of the Amsterdam criteria, and found 6 (67%) cancers in 4 families who showed a DNA replication error (RER+), but researchers failed to find any germline mutation in *hMSH2* and *hMLH1* in any patient. No alteration was found at the small repeated sequences in the gene for the type II receptor for transforming growth factor β (*TGFBR2*) in the tumor DNA. These results indicate that the carcinogenic process of familial gastric cancer may be different from that of HNPCC. According the study of the Finnish group,[30] most gastric cancer found in HNPCC is the intestinal type. In general, the frequency of intestinal type gastric cancer seems to be almost disappearing in the population under 50 years of age in Finland. Thus diagnosis of intestinal type gastric cancer at a young age may be a feature indicative of hereditary predisposition.[30] The Japanese series showed a similar trend, but we need to compare the data among the different cultures, based on the age and time specific prevalence, because our gastric cancers are frequently intestinal type. While gastric cancer is apparently one of the marker phenotypes of HNPCC, there seems to be other diverse genetic heterogeneities in familial gastric cancer.[27]

An attempt to identify HNPCC families from endometrial and ovarian cancer as proband cases was found less effective than the approach through the index case with colorectal cancer, because these 2 tumors occur much later (as much as 5 to10 years later than colorectal cancer in patients with HNPCC) (K. Ushio, oral communication, 1995). A family with an aggregation of transitional cell cancer of the urinary tracts was reported as a unique case of HNPCC.[31]

Association of adenomas

Woolf (1958) reported of a family with a high incidence of one or more small solitary colonic adenomas and of adenocarcinoma occurring in the members of single large kindred and concluded that a genetic determinant was responsible for the high incidence of isolated adenomas in this family.[32] There had been a long-time dispute whether such families had the genetically distinct syndrome of heritable solitary polyps[33] or if they were considered identical to those with cancer family syndrome.[34] A genetic linkage study showed that gene defect in the family with heritable solitary polyps is at a mutation in the *APC* gene.[35] It is now regarded as an attenuated form of familial adenomatous polyposis (AAPC) that is caused by mutation located very close to one another and nearer 5′ end of *APC*.[36]

HNPCC does not imply the absence of colorectal adenomas in this condition. As a matter of fact, there are studies indicating that common adenomatous polyps are more frequent in HNPCC than in the general population. Flat adenoma lesions, originally reported by Muto et al. in 1985,[37] were found in as often as 52% of the polyps taken from HNPCC family members and were predominantly distributed in the proximal colon, while overall frequency of flat adenoma among all polyps in the general population is 5.6%.[38] It is assumed that flat adenomas have a much higher malignancy potential than previously expected, since histologically benign-appearing flat adenoma with moderate atypia have already contained malignant DNA patterns, suggesting its important role in the genesis of small colonic carcinomas.[38] In the search for a phenotypic marker of HNPCC, Lynch et al. proposed that the presence of a flat adenoma might be a morphologic marker for identification of at least one subset of HNPCC, and they have referred to this condition as hereditary flat adenoma syndrome (HFAS).[39] The same group, however, later regarded some families with hereditary flat adenoma syndrome as identical with FAP, because they were associated with fundic gland polyps[40] and showed significant multipoint linkage with *APC*.[38] Watanabe et al., however, reported that RER+ had been found in 73.5% of flat adenoma associated with 9 families with HNPCC. Furthermore, in an analysis of the *APC* gene[41] no mutations were found in exons 1 to 6, in which mutations of *AAPC* were reported to exist.[37] These results strongly suggested that accurate diagnosis of patients with HNPCC requires careful clinicogenetic and molecular studies.

Other pathologic markers

Lectin UEA-1 that does not usually bind to the rectal mucosa showed positive binding by a significantly increased level in FH+. This differential lectin binding may be useful for presymptomatic diagnosis.[42]

Prognosis

Watanabe et al. found that the patients with FH+ should have a significantly improved survival rate when compared with the control group[43] and assumed that it could

be due to an increased proportion of tumor with inflammatory cell infiltration in patients with FH+. According to the nationwide colorectal cancer register report issued from JRSCC, edited by Koyama, the 5-year survival rate of 296 patients with FH+ was significantly better than that of patients with FH– (3620 cases) in every stage.[22] The continuous mutational load suffered by mismatch repair-deficient tumor cells might also cause them to have a relatively low fitness and, perhaps, to be more immunogenic. This could explain the relatively better prognosis of patients with HNPCC and patients with sporadic RER+ colorectal cancer.[44] Conversely, there were reports from the United States and Japan suggesting the defect of recognitive immunity in the families with HNPCC could be contributory to the genesis of the colon carcinoma.[45,46] This contradiction remains to be clarified.

Cancer Prevention Strategy

Case presentation

Family FC 5, with a pedigree covering 4 generations, was examined twice by the same investigators (Utsunomiya) with an interval of 8 years (Fig. 3).[10,47] This family typifies the diverse issues in management of HNPCC. The proband patient (case 1) was a woman aged 55 years who had undergone rectal excision for cancer at the age of 43, and colectomy for metachronous cancer at the age of 52. She visited us for a follow-up examination on her remnant colon, that had several polyps in 1981. Because of an extensive history of cancer in her family, her 4 children were examined by barium enema and colonoscopy. As a result, an advanced cancer was found in the sigmoid colon of one of them, a man aged 31 years (case 2). He had some episodes of blood staining in slender stools for several months but he was not concerned. A standard radical sigmoidectomy was performed to remove the lesion that was partly fixed to the bladder. The lesion was a diffusely infiltrating type (Fig. 4A, B). Histologic results showed the lesion was predominantly occupied by a well-differentiated adenocarcinoma partially mixed with a poorly differentiated or mucinous portion. Infiltration of fibrous tissue and lymphofollicular proliferation was predominant in the peripheral area. Lymphatic invasion was positive, but no metastatic foci were detected. His prognosis was definitely poor.[47] In 1989, he returned with cancer of the ascending colon and underwent curative surgery. When updating his family history we discovered that his mother (case 1) died at the age of 63 with cancer of the urinary bladder and 3 other members of his family had since had surgeries for colorectal cancer.[10] Germline mutation on *hMSH2* was detected in 2 of 3 asymptomatic children of the woman (case 2).

Family history taking

Case 2 is interesting because this patient seems to be one of the first patients in Japan in whom colorectal cancer was diagnosed through family history. However, these same researchers have not found this to be a frequent occurence. This fact suggests that there is a need for fundamental education on detailed family history taking that has been particularly neglected in Japanese surgical training.

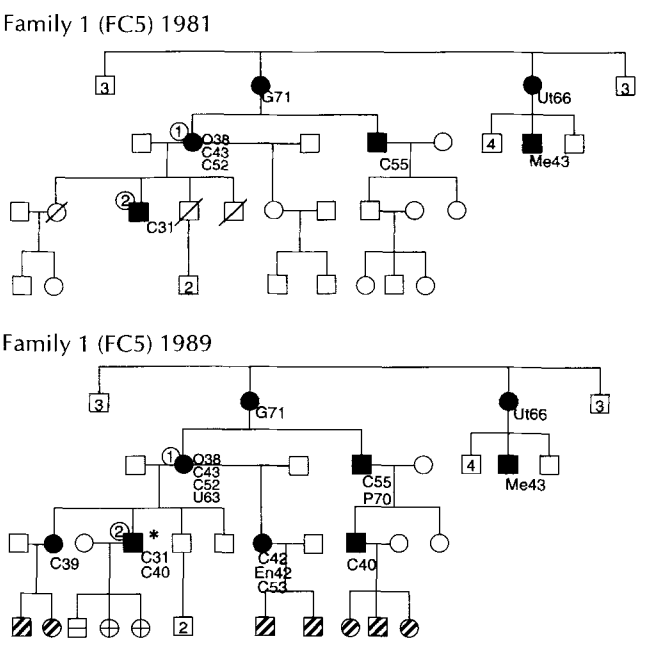

Fig. 3. Family 1 (FC5) suggesting the necessity of family surveillance (from Sakiyama et al.[10] and Ichikawa et al.[47]) Squares, men; circles, women; open symbols, no tumor; solid symbols, hereditary nonpolyposis colorectal cancer (HNPCC) patients with cancer; oblique closing line, negative by examination; diagonal stripes, not yet diagnosed; cross bars in Family 1 shows family members predisposed to HNPCC shown by presymptomatic DNA testing; horizontal bars, members without germline mutation shown by the DNA testing. Letters represent the site of cancer: C, colorectal; En, endometrial; Ut, uterine (site unknown); G, gastric; Me, mesenteric; O, ovarian; P, prostate; U, urinary tract. Numbers are age at diagnosis or death. Asterisk indicates the patient whose peripheral blood and/or cancer tissues were analyzed for mutation in *hMSH2* gene (see HNP1 in Table 9).

Mode of surveillance

In the first generation of family FC 5, there were 2 relatives who died of cancer between 1975 and 1976; one with gastric cancer and another with uterine cancer. They had survived to the age of about 70, and that was not much shorter than the average life span at that time in Japan. Apparently, genetic anticipation needs to be considered when deciding the age for starting surveillance. The seeming disappearance of gastric cancer and the increment of colorectal cancer seen as a typical generation trend of HNPCC may be accounted for in the priority site of the target organ for selective surveillance.

For an asymptomatic gene carrier, like the 2 daughters (7 and 12 years old) of our patient (case 2), intensive

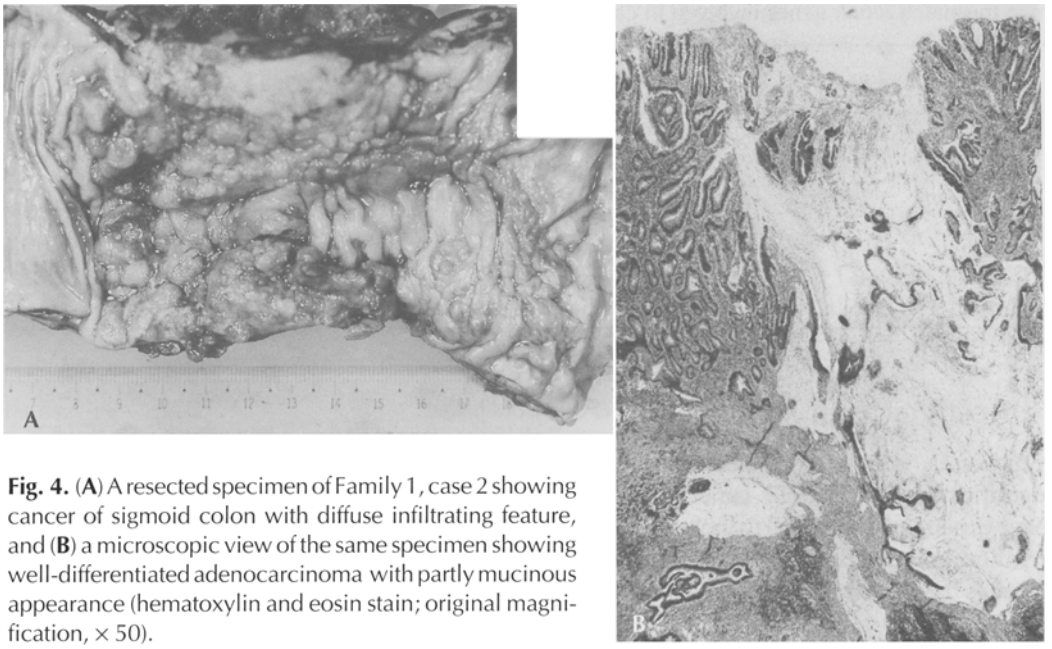

Fig. 4. (**A**) A resected specimen of Family 1, case 2 showing cancer of sigmoid colon with diffuse infiltrating feature, and (**B**) a microscopic view of the same specimen showing well-differentiated adenocarcinoma with partly mucinous appearance (hematoxylin and eosin stain; original magnification, × 50).

surveillance should be started at the age of 25 for the colon with a yearly total colonoscopy, and later surveillance on the endometrium.

For postoperative patients, surveillance should intensify with age. Our patient (case 2) had not received sufficient surveillance for 8 years after the operation because the responsible surgeon (JU) had changed his office location. This highlights the need to persist in follow-up at a local center for surveillance on patients with hereditary cancer.

Indication of prophylactic operation and surgical options

The course of the patient (case 1) showed that total colectomy may reduce the frequency of colectomy, but may not prevent her death from urinary bladder cancer. For initial rectal cancer, both a proctocolectomy with abdominal ileostomy and a conservative proctocolectomy (ileal-pouch anal canal anastomosis with stapler) could be justified at present. The former is, however, more appropriate, because in the patient who might be subsequently affected by cancer in the urinary bladder or in the endometrium, pelvic surgery will sacrifice 60 cm or more of the terminal ileum that may produce a disturbing high output ileostomy if the patient has an ileoanal pouch.

For asymptotic gene carriers, at this time, the commentary of Rodriguez-Bigas, that a prophylactic total abdominal colectomy in a completely normal colon as justified for familial adenomatous polyposis should not be recommended, even in mutation carriers, is supported by the authors.[48]

To benefit policy making for prophylactic surgery, a data base for age- and organ-specific cancer prevalence rate on HNPCC in Japan needs to be created.

STUDIES ON MOLECULAR FEATURES

A linkage study using microsatellite markers found that one of the genes causing HNPCC, autosomal dominant disease, is located at chromosome 2p, and that the patient's tumors had instabilities in the microsatellite region.[49] This phenotype was similar to those of *E. coli* and yeast mutants for mismatch repair genes—*mutL*, *mutS*, *PMS1*, *MLH1* and *MSH2*,[50] and subsequently, a human homologue of *MSH2*, *hMSH2* gene, was isolated from chromosome 2p in 1993.[51,52] This gene was confirmed to be the cause of HNPCC by the identification of germ line mutations in several HNPCC families. Since then, other human homologues of mismatch repair genes—*hMLH1*, *hPMS1*, and *hPMS2* have been identified and confirmed to be implicated in the development of HNPCC.[53,54] To date, nearly 200 germline mutations of these 4 genes have been detected in families with HNPCC throughout the world (Table 8).

In Japan, a collaborative study of hereditary colorectal tumor by clinical and basic researchers has continued since 1976, when a registry of FAP cases was established. During this period, researchers observed not only FAP, but also other types of hereditary colorectal tumors including HNPCC, Peutz-Jeghers syndrome, Turcot syndrome, and juvenile polyposis, and have collected pedigree data, as well as tumor and normal tissues from patients. The investigation was initially undertaken to analyze loss of heterozygosity (LOH) in these tumors using restriction fragment length polymorphism (RFLP) markers. Since the gene causing FAPs was identified in 1991, germline and somatic mutations of the *APC* gene have been extensively analyzed. The mechanism of carcinogenesis (adenoma-carcinoma sequence) in FAP patients, as

Table 8. Human mismatch repair genes involved in cancer.

Gene	Homologue		Function	cDNA (bp)	Protein (amino acids)	Chromosome
	E. coli	*S. cerevisiae*				
hMSH2	*mutS*	*MSH2*	Mismatch binding	3111	934	2p16
hMSH6 (GTBP)	*mutS*	*MSH6*	Mismatch binding	4200	1360	2p16
hMLH1	*mutL*	*MLH1*	Complex formation	2484	756	3p21.3–23
hPMS1	*mutL*	*PMS1*	Complex formation	3063	932	2q31–33
hPMS2	*mutL*	*PMS1*	Complex formation	2771	862	7p22

well as sporadic cases, is now more clearly understood. Since the identification of *hMSH2* gene in 1993,[51,52] researchers have expanded their focus to include HNPCC.

Both germline and somatic mutations of *hMSH2* and *hMLH1* genes have been detected in Japanese families with HNPCC,[55–57] and further analysis has shown a new type of HNPCC in families caused by the mutation of *hMSH6* mismatch repair gene.[58,59] In addition to these, the continuing study of carcinogenesis in HNPCC suggests a different mechanism from the adenoma-carcinoma sequence.[56] During these studies, a unique case of HNPCC, Turcot syndrome, was also observed.[60]

Germline and Somatic Mutations of *hMSH2* and *hMLH1* Genes in Japanese HNPCC Families

HNPCC is an autosomal dominant hereditary disease with high penetrance, usually resulting in colorectal cancer; it accounts for 5% of all colorectal cancers.[61] Extracolonic cancers, including endometrial, ovarian, gastric and other cancers, also affect family members with HNPCC. However, at this time, methods of detection and recognition of families with this disease have not been sufficiently developed; although, the Amsterdam minimum criteria for HNPCC have been established for comparative studies. This disease has no presymptomatic warning, and the cancer is usually found at an advanced stage in patients around 50 years of age. It has recently been suggested, however, that there may be a considerable number of families with HNPCC that do not fit these criteria, and they are difficult to diagnose clinically. It is important to identify the genetic changes inherited in both of these types of families with HNPCC for the prediction of susceptibility to cancer in family members, and also to discover unknown families that are predisposed to this disease.

Since the genes causing HNPCC (*hMSH2, hMLH1, hPMS1,* and *hPMS2*) have been isolated, it is necessary to determine which of these 4 genes is mutated and inherited in families with HNPCC that fit the minimum criteria. Germline mutations of *hMSH2* and *hMLH1* genes in 11 Japanese families clinically diagnosed as having HNPCC were examined, using a PCR-SSCP (polymerase chain reaction-single-strand conformation polymorphism) method and direct sequencing. Five of the 11 families (HNP Family 1–5 in Figs. 3 and 5 and Table 9) had *hMSH2* germline mutations.[55] In addition, 3 new families with HNPCC with germline mutations of *hMSH2* and *hMLH1* genes were found (HNP Family 7–9 in Fig. 5 and Table 9) after screening of 174 tumors by DNA analysis of microsatellite instability, followed by mutation analysis of mismatch repair genes and clinical investigation.[55] Two other investigations[57,62] have also detected 3 *hMSH2* germline mutations and 1 *hMLH1* germline mutation (Table 9). In addition to germline mutations, somatic mutations, or loss of normal allele of the same mismatch repair genes, were detected in tumors from patients in all of these families, as shown in Table 9, suggesting that 1 of the mismatch repair genes is inactivated in each HNPCC tumor.

The majority of germline and somatic mutations of *hMSH2* gene in Japanese HNPCC cases were frameshift and nonsense mutations, that led to stop codons. The deletions and insertions occurred at repeated sequences, such as TTT or AAAAA, that were similar to those observed in the *APC* gene. However, a general area of hot spots, as found in the *APC* gene, could not be determined with the *hMSH2* gene, and positions of truncation were scattered throughout the gene, except for the frequent mutation at the splice site of exon 5. Such characteristics are consistent with those of germline mutations of the *hMSH2* gene detected in other countries (Fig. 6). In the case of both *hMSH2* and *hMLH1* mutations, many missense types have been detected, but most frequently in *hMLH1*. Frameshift and nonsense mutation (that results in truncation of protein), and loss of entire sequence, can easily be understood to be an inactivative mutation of tumor suppressor genes. The mechanism of inactivation of genes through missense mutation, and the difference between missense mutation and genetic polymorphism, have not yet been fully clarified.

In the pedigrees of 6 families with HNPCC with germline mutations of the *hMSH2* gene (Family 1–5 and 7 in Figs. 3 and 5), 26 of the 30 patients (87%) had colorectal cancers with a mean age at diagnosis (or death) of 43 years of age (Table 10). Six of the 19

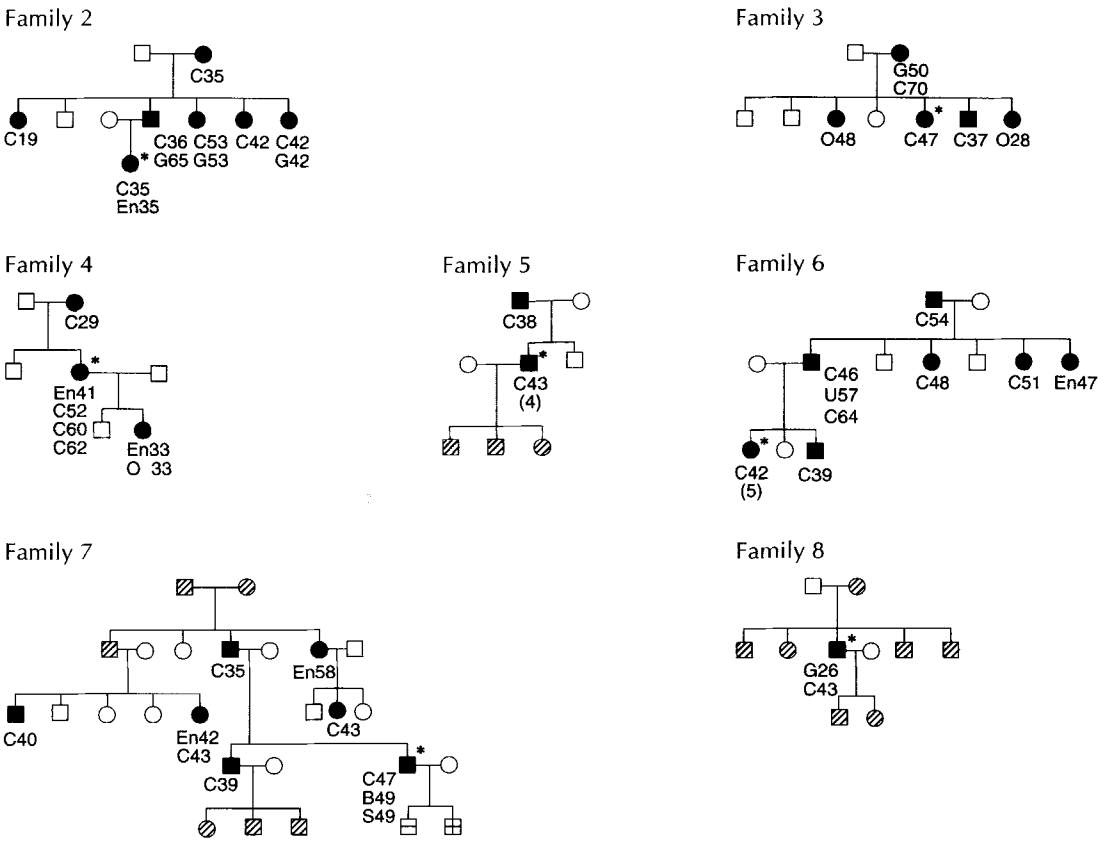

Fig. 5. The pedigrees of an additional 7 families with hereditary nonpolyposis colorectal cancer (HNPCC) with germline mutations of *hMSH2* and *hMLH2* genes that have been identified or presumed (from Miyaki et al.[55]). The symbols and labels are the same as those in Fig. 3. Numbers in parentheses are number of independent primary cancers that simultaneously existed.

Table 9. Germline and somatic mutations of *hMSH2* and *hMLH1* genes detected in Japanese families with HNPCC.

Histopathologic			Germline mutation			Somatic mutation		Ref
Family	Tumor	Type	Gene	Codon	DNA change	Codon	DNA change	
HNP1	CoCa	Invasive ca	*hMSH2*	136	TTT → TT	311–312	AACCTT → AACTT	55, 56
HNP2	EnCa	Endometrial ca	*hMSH2*	419	CAG → TAG	314	G/gta → G/gtt	55, 56
HNP3	CoCa	Invasive ca	*hMSH2*	811	TTA → TGA	Loss of normal allele		55, 56
HNP4	CoCa	Metastasized ca	*hMSH2*	227–229	AAAAAAA → AAAAAAAA	661	AAA → AAAA	55, 56
HNP5	CoCa1	Invasive ca	*hMSH2*	877–878	AGAGAG → AGAG	82–83	AAAA → AAAAA	55, 56
HNP5	CoCa2	Invasive ca	*hMSH2*	877–878	AGAGAG → AGAG	173–176	CT...GT → A (11-base pair deletion)	55, 56
HNP6	CoCa	Intramucosal ca	*hMSH2*		unidentified	182–188	TC...AG → TC...AGTC...AG (19-base pair repeat)	55, 56
HNP7	CoCa1	Invasive ca	*hMSH2*	314	G/gta → G/gtt	690–691	AAA → AA	55, 56
HNP7	CoCa2	Invasive ca	*hMSH2*	314	G/gta → G/gtt	311–312	AACCTT → AACTT	55, 56
HNP8	CoCa	Invasive ca	*hMLH1*	616–618	AAGAAGAAG → AAGAAG	Loss of normal allele		55, 56
HNP9	CoCa	Invasive ca	*hMLH1*	217	CGC → TGC	Unidentified		55
K41	CoCa		*hMSH2*	619	TAT → TAG	Loss of normal allele		57
K30	CoCa		*hMSH2*	836	TTC → TC	Unidentified		57
K39	CoCa		*hMSH2*	314	G/gta → G/gtt	Unidentified		57
JPN-1			*hMLH1*	582	CTC → GTC	Unidentified		62

HNPCC (and HNP), hereditary nonpolyposis colorectal cancer; CoCa, colon carcinoma; EnCa, endometrial carcinoma; ca, cancer; ref, reference number.

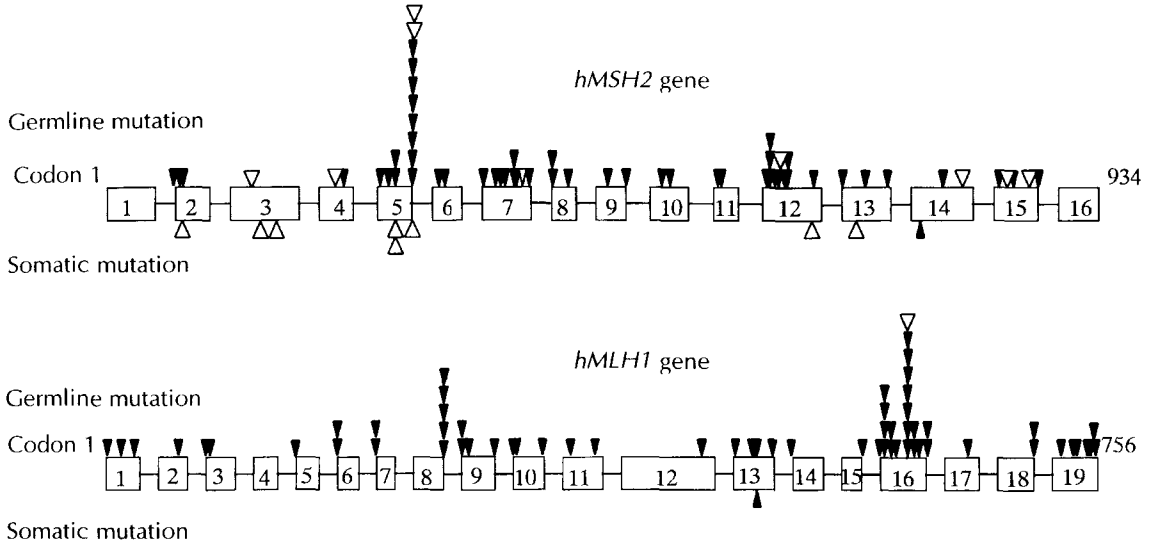

Fig. 6. The positions of germline and somatic mutations on *hMSH2* and *hMLH1* genes detected in patients with hereditary nonpolyposis colorectal cancer (HNPCC). Open triangles and open inverted triangles, detected in Japan; solid triangles and solid inverted triangles, detected in other countries. Missense mutations are not included.

women patients (32%) had endometrial cancer at about the age of 42. Four patients had both colorectal and endometrial cancers. These figures[55] are consistent with those recorded by Lynch.[61] In addition to these 2 most common types, there were ovarian (21%), gastric (14%), urinary tract (3%), prostate (9%), brain (3%), and skin (3%) cancers. In Family 2, a high incidence of gastric cancer was observed (3 of the 7 patients). In 14 cases (47%), a second, third, or even fourth primary cancer occurred in each patient. The patient in Family 8, whose germline mutation was identified in the *hMLH1* gene, had carcinoma of the ascending colon and also a gastric carcinoma; the patient in Family 9 with *hMLH1* germline mutation had 4 colorectal tumors. These extracolonic cancers, and the occurrence of multiple primary cancers, may also be helpful in making the diagnosis of HNPCC.[55]

Table 10. Tumors produced in 30 patients in 6 Japanese HNPCC families with *hMSH2* germline mutation.

Tumor	No. of patients (%)[a]	Age (y) at diagnosis (range)
Colorectal cancer	26/30 (87)	43 (19–63)
Endometrial cancer	6/19 (32)	42 (33–58)
Ovarian cancer	4/19 (21)	37 (28–48)
Gastric cancer	4/30 (14)	53 (42–65)
Urinary tract cancer	1/30 (3)	
Prostate cancer	1/11 (9)	70
Brain tumor	1/30 (3)	49
Skin cancer	1/30 (3)	49
Multiple cancer	14/30 (47)	

[a]The denominator differs in sex-specific disease, as there were 19 women and 11 men. Modified from Miyaki et al.[55]

Presymptomatic DNA Diagnosis of HNPCC

Typical HNPCC have a high penetrance within families, and clinical detection of patients with HNPCC is difficult due to no distinct presymptomatic warnings. Therefore, identification of germline mutation, as well as subsequent identification of patients, are both necessary.

After germline mutation was determined, presymptomatic analysis then became possible. For example, lymphocytes from the offspring of the patient, marked with an asterisk in Family 1, were diagnosed by means of PCR-SSCP analysis, as shown in Fig. 7. DNA from this patient showed an abnormal band, in the SSCP analysis, that was confirmed to correspond to the germline mutation of *hMSH2* gene (TTT to TT at codon 136). Of the 3 offspring, 2 daughters (7 and 12 years old) were found to have the same abnormal band corresponding to the germline mutation, that was confirmed by direct sequencing of DNA fragments in the abnormal band. This presymptomatic analysis could also detect an additional patient in Family 7. Such analysis is valuable for prediction and early detection of tumors so that treatment can be administered at an early stage.

Mechanism of Carcinogenesis in HNPCC: Replication Error and Genetic Alterations in HNPCC Tumors

DNA replication error in microsatellite loci (or microsatellite instability) is the most distinct feature of colorectal carcinomas from patients with HNPCC, which was a clue to the discovery of the causing genes for HNPCC.[49] RER(+) phenotype is assumed to be the result of the inactivation of a DNA mismatch repair gene by "two-

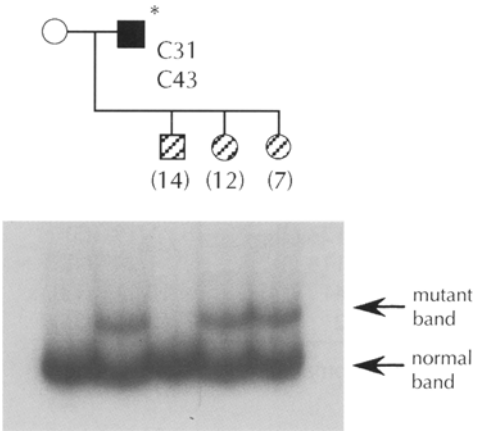

Fig. 7. An example of a presymptomatic DNA diagnosis of family members with hereditary nonpolyposis colorectal cancer (HNPCC) by polymerase chain reaction-single-strand conformation polymorphism analysis (from Miyaki et al.[55]). The mutant band corresponded to the germline mutation of *hMSH2* gene (TTT to TT at codon 136), which was confirmed by direct sequencing of DNA fragments extracted from the mutant band. Two daughters (7 and 12 years old) were found to have the same mutant band as that in the father with HNPCC, while a son (14 years old) had only the normal band.

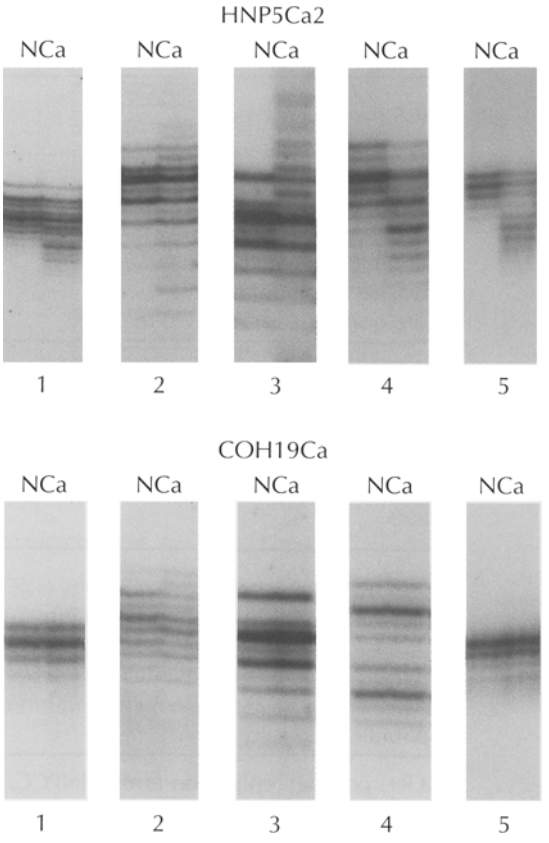

Fig. 8. Microsatellite instability in hereditary nonpolyposis colorectal cancer (HNPCC) colon carcinoma, HNP5Ca2, and sporadic carcinoma metastasized to the liver, COH19Ca (from Konishi et al.[56]). N, normal tissue; Ca, carcinoma; 1, D2S123; 2, D3S1029; 3, Mfd27; 4, Mfd47; 5, APΔ3. In HNP5Ca2, 5 of 5 loci are altered, and in COH19Ca, 1 of 5 loci is altered.

hit," since, in addition to the intrinsic germline mutation of mismatch repair gene, somatic alteration (mutation in the majority of cases and loss of normal allele in some cases) in the same gene were detected in HNPCC tumors, as indicated in Table 9.

To compare the extent of replication error between HNPCC and non-HNPCC cases, replication error was analyzed in 21 tumors from 12 unrelated patients with HNPCC, in 389 tumors from 57 patients with FAP, and in 206 sporadic colorectal tumors from 127 patients. These tumors were classified into 6 histopathologic types: hyperplastic polyp, moderate adenoma, severe adenoma, intramucosal carcinoma, invasive carcinoma, and carcinoma metastasized to the liver.[56] DNAs from these tumors were analyzed at 5 microsatellite loci: D2S123, D3S1029, Mfd27, Mfd47, and APΔ3 (Fig. 8).

As shown in Table 11A, a high frequency of RER(+) tumors were detected at all histopathologic stages in patients with HNPCC. The severe RER(+) phenotype was observed from the early stage in HNPCC, such as hyperplastic polyp[56] and also as flat adenomas.[41] In contrast, frequency of RER(+) tumors from patients with FAP and sporadic colorectal tumors was remarkably low in those with hyperplastic polyp, moderate adenoma, severe adenoma, and intramucosal carcinoma (0% to 5%), but it increased in stages, such as invasive carcinoma (13% to 24%) and in carcinoma metastasized to the liver (35%). These results suggest that RER(+) occurs from an early stage of carcinogenesis in HNPCC, but in later stages in non-HNPCC.

As to the number of altered loci (Table 11B), 100% of RER(+) tumors from patients with HNPCC exhibited 3 to 5 (severe RER+); however, in the patients with FAP and sporadic colorectal tumors, only 14% to 17% of the RER(+) tumors showed 3 to 5 (severe RER+), and 83% to 86% showed 1 or 2 (mild RER+). Generally, the fragment size in severe RER(+) tumors was significantly altered, while the fragment size in mild RER(+) tumors was slightly altered (Fig. 8). Although germline mutation was not detected, somatic mutations of *hMSH2* and *hMLH1* genes were detected in 1 of 3 patients with FAP and in 2 of 5 patients with sporadic colorectal tumors with severe RER(+) (Table 11B).[56] The loss of wild-type allele was also observed in these cases, which indicated that inactivation of DNA mismatch repair gene by "two-hit" is required for severe DNA instability, not only with HNPCC tumors, but also with non-HNPCC tumors, though less frequent in the latter cases. However, tumors with mild RER(+) from patients with FAP and sporadic colorectal tumors did not have mutations in *hMSH2* and *hMLH1* genes, therefore, mild RER(+) may be caused by other unknown mechanisms.

Table 11A. Frequency of microsatellite instability and histopathologic stage of colorectal tumors from HNPCC, FAP, and sporadic cases.

Histopathologic type	Tumors with instability/Tumors analyzed (%)		
	HNPCC	FAP	Sporadic
Hyperplastic polyp	1/1 (100)	0/1 (0)	0/2 (0)
Moderate adenoma	–	2/104 (2)	0/10 (0)
Severe adenoma	2/2 (100)	4/123 (3)	1/19 (5)
Intramucosal carcinoma	2/2 (100)	4/118 (3)	1/24 (4)
Invasive carcinoma	14/15 (93)	10/41 (24)	15/113 (13)
Carcinoma metastasized to the liver	1/1 (100)	1/2 (50)	13/37 (35)

HNPCC, hereditary nonpolyposis colorectal cancer; FAP, familial adenomatous polyposis. Modified from Konishi et al.[56]

Table 11B. Frequency of altered loci in RER(+) colorectal tumors from HNPCC, FAP, and sporadic cases.

	No. of tumors with RER(+) (%)	
	Altered loci/analyzed	
	1–2/5 (mild RER+)	3–5/5 (severe RER+)
HNPCC	0/20 (0)	20/20 (100)
FAP	18/21 (86)	3/21 (14)
Sporadic	25/30 (83)	5/30 (17)

RER+, positive replication error; HNPCC, hereditary nonpolyposis colorectal cancer; FAP, familial adenomatous polyposis. Modified from Konishi et al.[56]

Severe RER(+) resulting from inactivation of the mismatch repair gene has been reported to cause a high frequency of mutations in other mismatch repair genes having a repeated sequence, such as frameshift mutation at $(A)_8$ in *hMSH3*, and that at $(C)_8$ in *hMSH6* genes.[60,63,64] These mutations, in addition to inactivation of the genes causing HNPCC, may bring about genetic alterations in various cancer-related genes. To examine the extent of contribution of tumor suppressor genes and oncogene to HNPCC carcinogenesis, alterations were examined in several genes that were previously known to cause both FAP and sporadic colorectal cancer.[56] The somatic changes analyzed included mutations in *APC*, *TP53*, *K-ras*-2, *TGFBR2*, *BAX*, *E2F4* genes, and LOH on chromosomes 5q, 8p, 17p, 18q, and 22q. Somatic changes of these genes in RER(+) carcinomas are summarized in Fig. 9, including the frequency of somatic mutation in *hMSH2* and *hMLH1* genes. Frequencies of mutations in the *APC*, *TP53* and *K-ras*-2 genes in severe RER(+) HNPCC carcinomas were considerably low (0% to 22%), compared to those in mild RER(+) FAP and sporadic carcinomas (50% to 78%). The frequencies of LOH on chromosomes 5q, 8p, 17p, 18q, and 22q were also low (0% in RER(+) HNPCC carcinomas with germline mutation of *hMSH2* or *hMLH1* gene) compared to those in mild RER(+) FAP and sporadic carcinomas (53% to 86%). The frequencies of genetic changes in mild RER(+) FAP and sporadic carcinomas were similar to those in RER(–) FAP and sporadic carcinomas. In contrast, the frequency of mutation at $(A)_{10}$ repeating sequence in the *TGFBR2* gene was extremely high in RER(+) HNPCC carcinomas (78%) compared to the same mutation in RER(+) FAP and sporadic carcinomas (0% to 7%).[56,58,65,66] Frameshift mutation at $(G)_8$ of *BAX* gene and that at $(AGC)_{13}$ of *E2F4* gene also occurred in more than 50% of HNPCC carcinomas[58,60,67–69] but not in FAP and sporadic carcinomas.

In the adenoma-carcinoma sequence, mutation of the *APC* gene has been found at an early stage of carcinogenesis, and numerous somatic mutations have been detected in FAP and sporadic tumors. In patients with HNPCC, irrespective of the disease name, "nonpolyposis," a few tumors with *APC* somatic mutations were detected. This suggests that HNPCC carcinomas may also develop via adenoma, however, the frequency of *APC* mutation was much lower than in non-HNPCC cases. In contrast to this, the results in Fig. 9 suggest a larger contribution of *TGFBR* mutation than that of *APC* mutation in HNPCC. Furthermore, *TGFBR2* mutation was detected in hyperplastic polyps and adenomatous polyps from patients with HNPCC,[55,66] that suggests that this alteration may contribute to enhanced cell growth of HNPCC tumors from an early stage. In addition to *TGFBR2* mutation, *BAX* gene alterations, which may reduce apoptosis, and *E2F4* gene alterations, which may result in loss of cell-cycle regulation, seem to be important targets for severe RER(+) and may contribute to carcinogenesis in HNPCC.

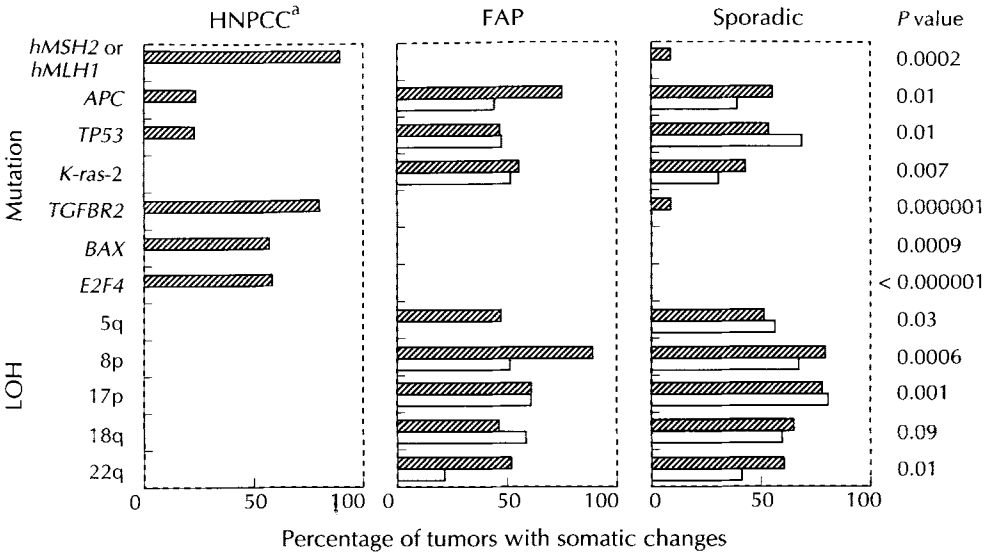

Fig. 9. Summary of frequencies of somatic changes in RER(+) and RER(–) colorectal carcinomas from hereditary nonpolyposis colorectal cancer (HNPCC), familial adenomatous polyposis (FAP), and sporadic cases (% of tumors with somatic changes) (from Konishi et al.[56]). *P* values were determined by Fisher's exact test, with respect to total RER(+) HNPCC carcinomas and RER(+) non-HNPCC carcinomas. LOH, loss of hetrozygosity. [a]With germline mutation of *hMSH2* or *hMLH1* gene. ▨, RER(+); □, RER(–).

These results showed different molecular features of HNPCC tumors from those of FAP and sporadic tumors, and suggest that a defect in DNA mismatch repair function does not largely contribute to previously known alterations of tumor suppressor genes, but results in somewhat different genetic changes from those in adenoma-carcinoma sequence. Further investigation of genetic alterations in other cancer-related genes, that have repeated sequence in coding and/or regulatory regions, is necessary to clarify the mechanism of carcinogenesis in HNPCC.

hMSH6 Gene Mutation as One of the Causes of HNPCC

Besides the 4 causative genes for HNPCC (*hMSH2*, *hMLH1*, *hPMS1*, and *hPMS2*), investigations in human cells and yeast have showed that *hMSH6*/GTBP is essential for mismatch repair function,[70] but the relationship of this gene to HNPCC was still unclear. A recent analysis of Japanese patients with HNPCC without germline mutation of *hMSH2* or *hMLH1* gene, has newly found an HNPCC family with germline mutation of *hMSH6* (Fig. 10).[58]

A carcinoma of the transverse colon, that formed in the patient marked with an asterisk in Family 13 (HNP13), at the age of 52, showed alterations in 5 of 7 dinucleotide repeat loci, and in 4 of 4 mononucleotide repeat loci. This carcinoma had several somatic alterations, such as mutations at $(A)_{10}$ of the *TGFBR2* gene and $(A)_8$ of *BAX* gene, and a missense mutation of the *APC* gene (different from the usual inactivating *APC* mutations that produce stop codons), but it did not exhibit loss of heterozygosity at chromosomes 5q, 8p, 17p, and 18q, nor mutation of *TP53* and *K-ras*-2 genes. Two small (2 mm) additional flat hyperplastic polyps in the same area showed RER(+) and *TGFBR2* mutation. An endometrial carcinoma from the same patient at age 53 also exhibited RER(+) at 3 of 4 dinucleotide repeats and at 3 of 4 mononucleotide repeats.

PCR-SSCP and direct sequencing analyses of these carcinomas and corresponding normal tissues showed a germline mutation of *hMSH6* gene: C deletion at codon 534, predicting a premature stop at codon 570 and truncation of the *hMSH6* protein (Table 12). The same germline mutation was also detected in the patient's sister who had an endometrial carcinoma at the age of 53; although 2 other sisters and 1 brother showed no evidence of tumors nor mutation. Two other sisters with endometrial or ovarian carcinoma were assumed to have the same mutation, as it is seen in their offsprings (Fig. 10). In addition to the germline mutation, somatic mutations of *hMSH6* gene were detected in both colonic and endometrial carcinomas from the original patient in Family 13 (Fig. 10 asterisk), both of which led to stop codons as indicated in Table 12. These somatic mutations were presumably in the allele without the germline mutation, suggesting that the inactivation of both allele of *hMSH6* was the cause of the RER(+) and the subsequent cancer.

This family with HNPCC with *hMSH6* germline mutation (Fig. 10) included patients with colonic, endometrial, ovarian, and pancreatic carcinomas similar to the usual manifestations of HNPCC in other families, although this family did not fulfill the Amsterdam criteria. It is notable, however, that endometrial and ovarian carcinomas were predominant in this family, in contrast

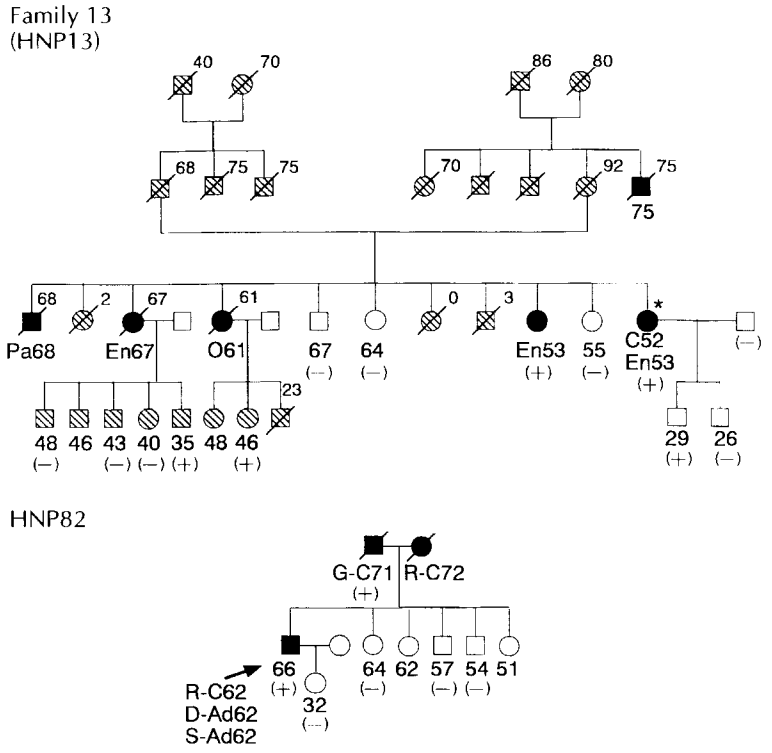

Fig. 10. Pedigrees of Japanese families with hereditary nonpolyposis colorectal cancer (HNPCC) with germline mutations of *hMSH6* gene (Family HNP13 from Miyaki et al.,[58] and Family HNP82 from Akiyama et al.[59]). Pa, pancreatic; En, endometrial; O, ovarian; C, colorectal; G, gastric; R, rectal; D, descending colon; S, sigmoid colon. Parentheses, results from *hMSH6* mutation analysis of blood DNA: (+), presence of the germline mutation; (–) absence of the germline mutation. Asterisk or arrow, the patient whose normal and tumor tissues were analyzed for mutation of *hMSH6* gene.

to the predominance of colorectal carcinomas in families with *hMSH2* or *hMLH1* germline mutation. The mean age of carcinoma formation in this family was 58 years old (58 y to 67 y) that is somewhat later than the mean age of 41 years old (19 y to 58 y) of first appearance of cancer in typical families with HNPCC with germline mutation of *hMSH2* or *hMLH1* (Table 10).[55] Such clinical aspects suggest that the effect of germline mutation of *hMSH6* gene on cancer pathogenesis may be different from that of *hMSH2* and *hMLH1* mutations.

Another germline mutation of *hMSH6* gene, a C insertion at codons 1085–1088, has also been detected in an HNPCC-like patient in Japan (Table 12).[59] The patient had rectal carcinoma and 2 colon adenomas at 62 years of age and a weak family history of gastrointestinal tumors (Fig. 10), indicating atypical HNPCC. Somatic frameshift mutations of the same gene were observed in the colorectal carcinoma and adenoma from the same patient (Table 12), indicating two-hit inactivation of the *hMSH6* gene. Microsatellite instabilities at mononucleotide repeats were detected in all tumors.

The existence of these 2 Japanese families suggests that there may be additional families with HNPCC with germline mutation of *hMSH6*, and more extensive analysis for this gene in patients with HNPCC is necessary to clarify the clinical effects of *hMSH6* mutation on tumorigenesis.

Turcot Syndrome: A Unique Case with Replication Error in Normal Tissue

Turcot syndrome is characterized by an association of malignant brain tumors and colon tumors. It has been suggested that there are 3 types of Turcot syndrome[71,72]: an autosomal dominant type associated with FAP, an autosomal dominant type associated with HNPCC, and a recessively inherited type. Although about 10 dominantly inherited cases with germline mutation of *APC* gene,[73,74] and 2 dominant cases with germline mutation of DNA mismatch repair genes,[74] have been reported as Turcot, the causative gene of recessive cases has not been identified, and the mechanism of carcinogenesis in every type of Turcot syndrome is still unclear.

A recent case of Turcot syndrome in Japan involved a patient that had no distinct family history of cancer in his parents (Fig. 11). This patient developed a grade IV astrocytoma at the age of 7 years, that recurred at 9 years, a malignant lymphoma at 15 years, a fibroma at 15

Table 12. Germline and somatic mutations of *hMSH6* gene in HNPCC tumors.

Histopathologic			Germline mutation			Somatic mutation		Ref
Tumor		Type	Gene	Codon	DNA change	Codon	DNA change	
HNP13	CoCa	Invasive ca	*hMSH6*	534	AAC → AA	128	CGT → CG	58
HNP13	EnCa	Endometrial ca	*hMSH6*	534	AAC → AA	1085–7	$(C)_8 \rightarrow (C)_7$	58
HNP82	CoCa	Invasive ca	*hMSH6*	1085–7	$(C)_8 \rightarrow (C)_9$	1085–7	$(C)_8 \rightarrow (C)_7$	59
HNP82	CoAd	Adenoma	*hMSH6*	1085–7	$(C)_8 \rightarrow (C)_9$	1085–7	$(C)_8 \rightarrow (C)_7$	59

HNPCC (and HNP), hereditary nonpolyposis colorectal cancer; ca, cancer; ref, reference.

years, 3 independent colon carcinomas and nearly 10 colon polyps in the period between the ages of 13 and 16 years. The patient, who had suffered from slight mental retardation, died at 16 years of age. Genetic changes were analyzed in 6 tumors, including: 1 astrocytoma (As) , 2 carcinomas of the sigmoid colon (CoCa1 and CoCa2), 1 carcinoma of the ascending colon (CoCa3), and 2 tubular adenomas of the colon (CoAd1 and CoAd3).[60]

As indicated in Table 13, all 6 tumors exhibited obvious alteration at 3 or more microsatellite regions. Moreover, colon adenomas and carcinomas had alterations at repeated sequences in *TGFBR2*, *hMSH3* and/or *hMSH6* genes, and 2 of 3 carcinomas had alterations at $(AGC)_{13}$ in *E2F4* gene. It was further tested to see if somatic mutation of *APC*, *TP53*, and *K-ras* genes occurred in these Turcot tumors at a high frequency, as in non-HNPCC, or at a low frequency, as in usual HNPCC. All 3 colon carcinomas showed somatic mutations of *APC* gene, that could have possibly resulted from very severe replication error. With respect to the *TP53* gene, the astrocytoma had a mutation at codon 273, and 2 colon carcinomas both showed 2 mutations in this gene. Three of these 4 mutations have not previously been detected in colorectal carcinomas, and the presence of 2 mutations without allele loss in 2 colon carcinomas was a different pattern from the usual *TP53* alteration (mutation plus loss) in non-HNPCC carcinomas. Such a high frequency of *APC* and *TP53* gene mutation has not previously been observed in usual HNPCC carcinomas with germline mutation of *hMSH2* or *hMLH1* gene.[56]

To examine why this patient developed multiple tumors in the colon and other tissues at an early age, and

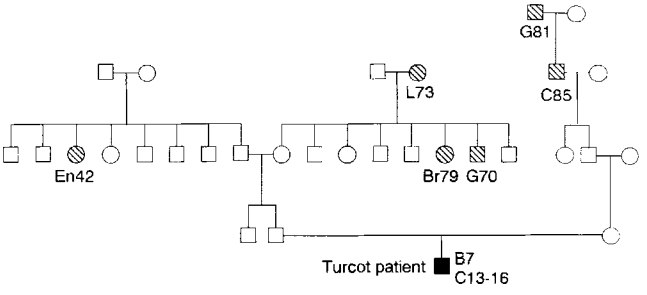

Fig. 11. The pedigree of a Japanese patient with Turcot syndrome (unpublished data from Junko Nishio). Letters represent the site of cancer: B, brain; Br, breast; C, colon; En, endometrial; G, gastric; L, liver. Numbers are the age at diagnosis.

Table 13. Somatic genetic alterations in brain and colorectal tumors from a patient with Turcot syndrome.

Tumor[a]	Site	Size (mm)	Age at diagnosis	Somatic mutation[c]							
				RER[b]	*hMSH3* $(A)_8$	*hMSH6* $(C)_8$	*TGFBR2* $(A)_{10}$	*BAX* $(G)_8$	*E2F4* $(AGC)_{13}$	*APC* (codon mutation)	*TP53* (codon mutation)
As	Brain	(4)	9 y	3/5	–	–	–	–	ND	ND	+(273 CGT to TGT)
CoAd1	Colon	(15)	13y11m	4/5	+[d]	+[d]	+[e]	–	ND	ND	–
CoAd3	Colon	(10)	15y3m	4/5	–	–	+[f]	–	–	–	–
CoCa1	SC	(40)	13y8m	5/5	+[d]	–	+[d]	–	–	+(1450 CGA to TGA)	–
CoCa2	SC	(60)	15y9m	5/5	–	+[f]	+[d]	–	+[g]	+(847–8 A ins) +(1555 A ins)	+(146 G del) +(234 TAC to CAC)
CoCa3	AC(23)	16 y	4/5	+[e]	–	+[d]	–	+[h]		+(1462–5 AG ins) +(1508 GCT to GTT) +(880 ATC to ACC) +(890 GTC to ATC)	+(157 GTC to ATC) +(213 CGA to TGA)

RER, replication error rate; ins, insertion; del, deletion; SC, sigmoid colon. [a]As, astrocytoma; CoAd, colon adenoma; CoCa, colon carcinoma; [b]number of altered microsatellite loci/analyzed (primers used were D2S123, D3S1029, Mfd27, Mfd47, and IL2RB); [c]+, mutation was detected; –, mutation was not detected; [d]1–bp deletion; [e]1–bp insertion; [f]2–bp insertion; [g]3–bp insertion; [h]3–bp deletion; ND, not done. Modified from Miyaki et al.[60]

why these tumors had such drastic genetic alterations, replication error in normal tissues from this patient were examined and compared to that in normal tissues from HNPCC patients with germline mutation in *hMSH2* or *hMLH1* genes. Products of 16 to 64 parallel polymerase chain reactions, containing primers for each microsatellite marker and each diluted DNA sample from normal tissues were electrophoresed. As indicated in Table 14, DNA from normal colon mucosa of this patient showed a high frequency (53%) of DNA instability, compared with the absence of such high instability in known patients with HNPCC. Cultured normal skin fibroblasts and normal brain tissue of this patient also showed similar instability (22% to 23%). However, lymphocytes from both of the patient's parents and known patients with HNPCC did not show a high instability. Such high DNA instability in normal tissue has been reported in cases of knock-out mice with homologous mutation of *PMS2* or *MSH2* gene, and in cases of patients with HNPCC with a germline mutation of *hPMS2* gene; but whether these cases of HNPCC were Turcot syndrome or not has not been clarified.

In this Turcot patient and his parents, no germline mutation was detected in coding regions of *hMSH2*, *hMLH1*, *hPMS1*, *hMSH6*, and *hTV1* genes, with the exception of a germline mutation of CAG(Glu) to AAG(Lys) at codon 705 of the *hPMS2* gene inherited from his mother, which may not be polymorphism, as it has not been detected in 80 other individuals. One somatic mutation at the promoter region of *hPMS2* gene (a C deletion at nucleotide −123 to −120 upstream of the codon 1) was detected in 1 colon carcinoma (CoCa2).

Table 14. Frequency of microsatellite instability in diluted DNA of normal tissues from Turcot and HNPCC patients (% of PCR reaction exhibiting alterations in microsatellite regions).

Patient	Tissue	PCR with alteration/PCR with visible bands (%)		
		D2S123	D3S1029	NEFL
Turcot patient	Normal mucosa	36/53 (68)	10/27 (37)	ND
	Normal skin fibroblasts	10/47 (21)	7/29 (24)	ND
	Normal brain tissue	2/9 (22)	ND	ND
Mother of Turcot patient	Normal lymphocytes	0/18 (0)	0/19 (0)	ND
Father of Turcot patient	Normal lymphocytes	0/22 (0)	2/19 (11)	0/23 (0)
HNPCC patient (*hMSH2*, codon 811 TTA to TGA)	Normal mucosa	1/25 (4)	0/15 (0)	1/14 (7)
HNPCC patient (*hMSH2*, codon 136 T del)	Normal skin fibroblasts	0/23 (0)	0/20 (0)	ND
HNPCC patient (*hMLH1*, codon 616-8 AGG del)	Normal mucosa	0/22 (0)	0/15 (0)	0/14 (0)
FAP patient	Normal mucosa	1/21 (5)	0/20 (0)	ND
Sporadic CRC patient	Normal mucosa	1/20 (5)	0/12 (0)	ND

To examine the DNA in individual cells of normal tissues, DNA samples were diluted to such a concentration that some fractions exhibited absence of band or one band corresponding to one allele in polymerase chain reaction (PCR) reactions (3 to 30 pg in each PCR reaction). Products of 16 to 64 PCR reactions, containing primers for each microsatellite marker and each diluted DNA sample were electrophoresed. The percentages of cells with microsatellite alterations were assessed as the number of PCR reactions exhibiting abnormal bands divided by the number of PCR reactions exhibiting visible bands (normal or abnormal) in electrophoresis. Accordingly, percentages are not exact contents of cells with alteration, but relative values. HNPCC, hereditary nonpolyposis colorectal cancer; FAP, familial adenomatous polyposis; CRC, colorectal cancer; ND, not done. Modified from Miyaki et al.[60]

The effects of these germline and somatic alterations in *hPMS2* gene, however, are unclear as yet. This germline mutation of *hPMS2* alone may not be the cause of Turcot syndrome, because his mother had no tumors, and her normal lymphocytes did not show the same DNA instability seen in her son.

This Turcot patient may be a unique case, compared to the usual HNPCC patient, and the high DNA instability in normal tissues suggests that the patient had a homozygous recessive mutation of a gene, including the possibility of *hPMS2*. Alternatively, this patient may be a compound heterozygote for mutations in a mismatch repair gene, or a heterozygote for other unknown mismatch repair gene mutation with a strong dominant-negative effect. In either case, it may be that the RER(+) phenotype causes replication errors rapidly and simultaneously in other mismatch repair genes and cancer-related genes, and may result in the very early development of malignant brain tumors, colorectal tumors, malignant lymphoma, and fibromas, as in the cases of homologously deficient mice. Further studies are necessary to clarify the germline mutation of this patient, and to detect genetic alterations in carcinomas from other patients with Turcot, to fully understand the mechanisms of carcinogenesis in this syndrome.

CONCLUSION

Study on hereditary tumors has accelerated cancer research in 2 major ways; for understanding molecular etiopathogenesis of cancer, and for creating cancer prevention strategies. Research in these 2 areas has had fruitful results, particularly in colorectal cancer.

Recently the responsible gene for Peutz-Jeghers syndrome has been identified as *STK11*,[75] which was previously registered as 1 of the serine/threonine kinase genes, *LKB1*, by Nezu of Chugai Pharmaceutical Institute. This is a significant event in hereditary colorectal cancer research that followed the previous accomplishment on FAP in 1991, on HNPCC in 1993, on Juvenile polyposis, and Cowden disease in 1997 (Table 2). Now, the genotypic background of clinical classification of hereditary colorectal cancer seems to be completed (Table 2). McKusick[76] has further classified that HNPCC that includes the form due to mutations in *MSH2* be referred to as type 1 (HNPCC1); those due to mutations in *MLH1* be referred to as type 2 (HNPCC2); those due to mutations in *PMS1* as type 3 (HNPCC3); those due to mutations in *PMS2* as type 4 (HNPCC4); and those due to mutations in *MSH6* (GTBP) as type 5 (HNPCC5). Japanese investigators have made a considerable contribution to colorectal cancer research as evidenced by the findings in FAP, HNPCC5, and in Peutz-Jeghers syndrome.

In the beginning of 1970 we established the Polyposis Center, which has registered almost all types of hereditary colon cancer. At that time, however, we hardly believed the genes responsible for these cancers could be identified within this century, but today no one doubts that the successful interpretation of the whole story of genetic changes in colorectal cancer will be achieved within the next few years.

We must recognize the serious problems in the Japanese educational system on genetics, particularly concerning clinical and epidemiologic aspects. To cope with this revolutionary change, we must urgently review the way that cancer genetics is taught in Japan. From over 20 years' experience at the Polyposis Center, and from the UICC symposium on Familial Cancer and Prevention, we have come to believe that our highest priority is to accelerate the training of specialized cancer health providers who must understand genetic principles to carry out the difficult task of analyzing a pedigree of many generations, be able to perform continuous surveillance and counseling of the patients and their family members, while adhering to the guidelines for ethical, legal, psychological, and social issues. Without this specialized knowledge, there will be no one to monitor the life-long natural course of gene carriers, and to assess their behavioral and psychological reactions to the impacts of genetic testing.

In a cooperative effort, we initiated the Familial Cancer Society in 1995, and are now ready to present the guidelines for ethical, legal, psychological, and social issues. The next function of the Society is a series of seminars on cancer genetic counseling for health providers, to be initiated this year. We believe that creating personal communication among specialists with the same goal in mind is the most effective way of constructing a cancer genetic network with multiple nodal points throughout the nation. This network will be essential to implement a cancer genetic strategy. HNPCC and FAP provide an appropriate model to test this system.

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