Lynch syndrome: History and current status

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1. Introduction

The annual worldwide incidence of colorectal cancer (CRC) is 944,717 with a mortality of 492,411 [1]. Approximately 5% to 10% of this total CRC burden is due to primary Mendelian inheritance factors, making hereditary CRC a major public health problem throughout the world. These estimates of the hereditary burden of CRC may be conservative when considering the existence of low-penetrant genes such as the Ashkenazi I1307K mutation [2], the recently described autosomal recessive form of familial adenomatous polyposis (FAP)-like families due to the MYH mutation [3, 4], and the hereditary breast and colon cancer (HBCC) syndrome [5,6].

Understanding the role of genetics in the etiology of CRC has increased rapidly during the past decade, due to the prodigious advances in molecular genetics [7]. Indeed, this information has evolved so rapidly that it has outpaced the ability of physicians to keep abreast of these fast-breaking events.

2. Spectrum of hereditary forms of CRC

Figure 1 depicts the relative occurrence rates of sporadic, familial, or hereditary classifications of CRC. These estimates of the familial and hereditary categories of CRC risk are probably highly conservative, given the fact that those extracolonic cancers that are integral lesions to specific hereditary CRC syndromes are often not given due consideration when evaluating pedigrees and thereby calculating estimates of the hereditary burden of CRC. The very terms "sporadic," "familial," and "hereditary" have limited meaning. For example, "sporadic" CRC (a single case of CRC) and

"familial" CRC (two or more first-degree relatives with CRC) are relatively crude terms. Specifically, they do not take into consideration factors that may obfuscate, and thereby underestimate, the true incidence rate of the genetic susceptibility to CRC. These factors include the presence of cancer of other organ sites, low-penetrant mutations, possible autosomal recessive inheritance, the general lack of genetic informativeness which may occur in a small family, adoption, false paternity, outright denial, and/or the lack of cooperation of family members and even their physicians.

3. Proximal vs. distal hereditary CRC

Hereditary CRC-prone syndromes can be divided into those with proximal predilection in the colon and those with distal predilection in the colon, based upon molecular genetic features. Specifically, tumors, "... that exhibit microsatellite instability (MIN) tend to occur in the right colon, have diploid DNA, carry characteristic mutations (transforming growth factor β Type II receptor, *BAX*) and behave indolently. Hereditary non-polyposis colorectal cancer (HNPCC) [or Lynch syndrome] epitomises this route of tumour development. Conversely, tumours with chromosomal instability (CIN) tend to be left-sided, have aneuploid DNA, carry characteristic mutations (*K-ras, APC, p. 53*) and behave aggressively. Familial adenomatous polyposis (FAP) epitomises this type of tumour." [8] Considerable embryologic, vascular, and molecular evidence has merged showing that there are two colons: a right colon and a left colon [9,10].

Our purpose is to describe the history of the Lynch syndrome, which is the most common form of hereditary CRC, with conservative estimates that it accounts for about 2% to 7% of the total CRC burden [11].
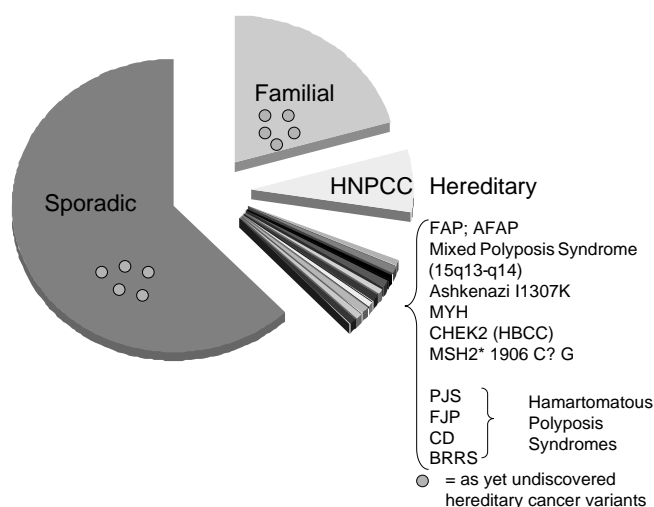
Fig. 1. Circle graph showing relative numbers of colorectal cancer cases that are considered sporadic, familial, or due to a recognized hereditary cancer syndrome. (Reprinted with permission from Lynch et al. *Cancer* 2004;100:53–64.) Abbreviations: HNPCC = hereditary nonpolyposis colorectal cancer FAP = familial adenomatous polyposis AFAP = attenuated familial adenomatous polyposis PJS = Peutz-Jeghers syndrome FJP = familial juvenile polyposis CD = Cowden's disease BRRS = Bannayan-Ruvalcaba-Riley syndrome.

4. History of HNPCC (Lynch syndrome)

In 1962, one of the authors (Henry Lynch, M.D.), who at that time was a resident in internal medicine, had a patient who was recovering from delirium tremens. When queried about his reason for excessive drinking, the patient's response was that he knew that he, like "everyone" in his family, was going to die of cancer, and that it would likely be cancer of the colon, although he realized that many other cancers had also affected the family. Shortly thereafter, he was diagnosed with adrenal cortical carcinoma. He was extremely helpful in allowing Lynch to compile his family history. With the capable help of Ann Krush, M.S.W., they began contacting the proband's relatives, many of whom were farmers residing in Missouri, Kansas, and Nebraska. The cooperation from the family members was superb. Many reported a personal history of cancer, particularly involving the colon and often with early age of onset. A significant number had had multiple primary cancers, including metachronous CRCs and, among the women, CRC often associated with endometrial and ovarian carcinoma.

In a review of the literature, Lynch and Krush did not find evidence for a hereditary basis for this pattern of multiple primary tumor combinations. They wondered whether they might be dealing with a "new" hereditary cancer syndrome. This kindred was labeled Family N, for "Nebraska." (The *MSH2* R680X germline mutation was subsequently identified in Family N in the year 2000.)

These investigators spent countless weekends during the early 1960s going from hospital to hospital, particularly in agricultural communities in northwest Missouri where many of the members of Family N resided, collecting details of medical and pathology records so that the pedigree could be documented as thoroughly as possible. Most noteworthy was the fact that patients in this family who manifested colorectal carcinoma did not have multiple colonic adenomas. This was important at that time, because it was believed by many clinicians and geneticists that the only hereditary CRC disorders were those associated with evidence of multiple colonic polyps, such as FAP.

The research team was joined by Charles W. Magnuson, M.D., (now deceased), a gastroenterologist. The local hospitals provided access to their outpatient facilities and weekends were spent educating the family members, performing physical examinations, and obtaining biospecimens for future study. Additional information about their family history of cancer was accrued through detailed questionnaires for the extended family, and goals for this family study were reinforced. During these family information sessions (FISs) [12], family members often stated that, for the first time in their lives, health care professionals had expressed intense interest in the cancer occurrences in their family, and were attempting to find answers to this familial cancer puzzle. Family N showed a well-defined autosomal dominant mode of genetic transmission of carcinoma of the colon with early age of cancer onset,

multiple primaries, and with the integral association of carcinoma of the endometrium and ovary.

Cancer at additional anatomic sites are now known to be part of the Lynch syndrome [13,14].

In 1964, an abstract about Family N was submitted to the American Society of Human Genetics. It was accepted and the first scientific report of Family N was presented at the annual meeting of this organization. During the presentation, Marjorie W. Shaw, M.D., a geneticist at the University of Michigan School of Medicine in Ann Arbor, asked some pertinent questions, among which was whether the findings in the family could be attributed, in part, to cytoplasmic inheritance. Given the temporal setting of the early 1960s, this was one of the etiologic hypotheses regarding cancer genetics at the infrahuman level. Specifically, there was curiosity about the role of mitochondria, which especially interested Dr. Shaw because of her prior work in plant genetics. She also told the audience that she had a family from Michigan that was similar to Family N, which was subsequently labeled Family M, for "Michigan," and she invited Lynch to collaborate with her in studying this family. Lynch and Krush then made multiple additional visits to Ann Arbor to study Family M with Shaw. During this timeframe, the developing research team was joined by a pathologist, Arthur L. Larsen, M.D. (now deceased), who meticulously reviewed all of the available pathology on Families N and M. Collectively, this work culminated in the first publication on the subject in 1966 [15].

The possibility of an oncogenic virus in interaction with a germline bmutation was also considered in the syndrome's etiology [15]. For example, given the research climate of the 1960s and the early 1970s, the cancer susceptible genotype in Families N and M and, subsequently, Family G (discussed below), was considered by the Lynch research team to be interacting with environmental carcinogens such as an oncogenic virus [15,16]. It was postulated that such a cancer-causing virus, possibly prevalent in the general population, would prove particularly virulent to individuals with a cancer susceptible genotype. It was reasoned that if this virus was an exogenous carcinogen, it most likely would be interacting with a cancer susceptibility genotype. On a priori grounds, such an agent would most likely be ubiquitous, but would cause cancer in only a subset of highly genetically susceptible humans.

The question was then raised as to whether such an oncogenic virus, similar to the polyoma virus in mice [17], might be interacting with the cancer-prone genotype in these families. Thus it was inferred that in dominantly inherited cancer, it is not outlandish to suppose that a viral gene has become "integrated" into one of the chromosomes of the host, thereby explaining the transmission of susceptibility from one generation to the next. Furthermore, it was inferred that if viral oncogenes were ubiquitous in the environment, then everyone would have been exposed to them, but an unknown event would be required for the onset of cancer in an individual who has not acquired the oncogene. It was believed that this might have been a low-probability event, but that the phenotype (cancer) might have increased with intensified exposure to the virus [15].

5. Family G

The first study of a family that represented what is now known as HNPCC began in 1895, when Aldred Warthin, M.D., a renowned pathologist, learned that his seamstress was depressed because she was convinced, based on her family history, that she would one day die of cancer of the female organs or bowels. When queried about this problem, the seamstress told Warthin that it was inevitable that she would die early in life because "Everyone in my family dies of those cancers." Just as she predicted, she died at an early age of metastatic endometrial carcinoma. Warthin published a description of this family, which he called Family G, in 1913 [18]. Members of Family G had migrated to Michigan from southern Germany during the early and mid-1880s.

A. James French, M.D., chairman of pathology at the University of Michigan School of Medicine during the 1960s, learned about Lynch's study of cancer families and invited him to update the study by his predecessor Dr. Warthin. Dr. French provided Lynch with all of Warthin's meticulous pathology, clinical, and genealogical documentation of Family G.

In addition to intensively studying the family in Michigan, Lynch and Krush also visited southern Germany and investigated those members of Family G who had not emigrated to the United States. The update of Family G that resulted from these studies was published in 1971 [16]. In testament to the progress that has been made in the field of cancer genetics, the *MLH2* germline mutation responsible for cancer predisposition in Family G was subsequently identified through conversion technology in 2000 [19].

Table 1
Landmarks of Lynch Syndrome History

Feature	First report	References
Family G of Warthin (study began 1895)	1913	[18]
Genetic Counseling	1965	[118,119]
First report of Lynch et al. on Families N and M	1966	[15]
Early age of cancer onset	1966	[15]
Autosomal dominant inheritance pattern	1966	[15]
Family information session (FIS)	1966	[12,15,120]
Screening recommendations	1967	[21]
Update of Family G	1971	[16]
Proximal colon involvement	1977	[22]
Beginning of study of Lynch syndrome in Uruguay	1977	[84]
Recommendation of prophylactic TAH-BSO	1978	[121]
Muir-Torre syndrome (as variant of Lynch syndrome)	1980	[38,122]
Increased incidence of synchronous and metachronous CRC	1982	[27,28]
Lynch syndrome studies begin with the Navajo	1983	[96,99,123–126]
Tritiated thymidine distribution studies of rectal mucosa	1983	[23]
HNPCC named "Lynch syndrome"	1984	[127]
Selenium levels in Lynch syndrome studied	1984	[128]
Formation of ICG-HNPCC	1989	[24,129]
Lectin binding studies in FAP and HNPCC	1990	[130–132]
Amsterdam I criteria	1991	[24]
Accelerated carcinogenesis and interval CRC	1992	[31,36,37,43,44]
First cancer susceptibility locus found on 2p through linkage analysis	1993	[56]
Second cancer susceptibility locus found on 3p through linkage analysis	1993	[57]
DNA mismatch repair genes reported	1993	[62–65]
RER+ (MSI) phenotype described	1993	[58]
Germline mutations in the syndrome	1993	[62]
MSH2 mutation identified	1993	[63]
Extracolonic adenocarcinomas	1994	[14]
Distinctive pathology features	1994	[31]
MSH2; *MLH1* mutations	1994	[64,65]
Creighton group's involvement in Uruguayan study	1995	[87]
Historical perspective through 1995	1995	[133]
Role of DNA MMR genes in CRC tumorigenesis	1995	[134,135]
Recommendations of prophylactic subtotal colectomy	1996	[108,109]
Survival advantage	1996	[29,30]
NIH NCI workshop on HNPCC (Bethesda Guidelines)	1996	[26]
MSH6 mutation	1997	[72,136]
NIH NCI update on MSI	1997	[137]
Small bowel involvement	1998	[138]
Founder mutation in Finland	1998	[90]
Amsterdam II criteria	1999	[25]
Tumor infiltrating lymphocytes and their association with MSI	1999	[32]
Conversion technology	2000	[19]
A complex mutation of *MLH1* at codon 222 is associated with adolescent onset of CRC (more early onset CRC families needed for study)	2001	[139]
Fluorouracil-based adjuvant chemotherapy benefits patients with stage II or stage III CRC with MSS or MSI-L tumors but not those with MSI-H tumors	2003	[60]
H(2)O(2) effect improves survival in DNA MMR-deficient cell line	2003	[140]
MSH2 del1-6 founder mutation in the United States	2003	[100]

6. Study of other "Cancer families"

Lynch began studying other CRC-prone families and also found families in the literature whose clinical findings were suggestive of a hereditary cancer-prone syndrome [20,21]. In 1971, based on these family studies, Lynch and Krush [20] reported the criteria for the "cancer family syndrome," namely, increased incidence of adenocarcinoma, primarily of the colon and endometrium, increased frequency of multiple primary malignant neoplasms, early age of onset, and autosomal dominant inheritance [21]. Further study allowed Lynch et al. [22] in 1977, to add the observation that the CRCs in Lynch syndrome occur with significantly greater excess in the proximal colon with about one-third of them occurring in the cecum. Table 1 provides

Table 2
Amsterdam I and Amsterdam II Criteria, and Bethesda Guidelines

Amsterdam I criteria [24]:
– At least 3 relatives with histologically verified colorectal cancer:
1. One is a first-degree relative of the other two;
2. At least two successive generations affected;
3. At least one of the relatives with colorectal cancer diagnosed at <50 yrs. of age;
4. familial adenomatous polyposis has been excluded.

Amsterdam II criteria [25]:
– At least 3 relatives with an hereditary nonpolyposis colorectal cancer-associated cancer (colorectal cancer, endometrial, stomach, ovary, ureter/renal pelvis, brain, small bowel, hepatobiliary tract, and skin [sebaceous tumors]):
1. One is a first-degree relative of the other two;
2. At least two successive generations affected;
3. At least one of the hereditary nonpolyposis colorectal cancer-associated cancers should be diagnosed at <50 yrs. of age;
4. familial adenomatous polyposis should be excluded in any colorectal cancer cases;
Tumors should be verified whenever possible.

Bethesda Guidelines for testing of colorectal tumors for microsatellite instability [26]
1. Individuals with cancer in families that meet the Amsterdam Criteria
2. Individuals with two HNPCC-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers*
3. Individuals with colorectal cancer and a first-degree relative with colorectal cancer and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma; one of the cancers diagnosed at age <45 y, and the adenoma diagnosed at age <40 y
4. Individuals with colorectal cancer or endometrial cancer diagnosed at age <45 y
5. Individuals with right-sided colorectal cancer with an undifferentiated pattern (solid/cribiform) on histopathology diagnosed at age <45 ÿ*
6 Individuals with signet-ring-cell-type colorectal cancer diagnosed at age <45 ÿ***
7. Individuals with adenomas diagnosed at age <40 y

*Endometrial, ovarian, gastric, hepatobiliary, or small-bowel cancer or transitional cell carcinoma of the renal pelvis or ureter.
**Solid/cribiform defined as poorly differentiated or undifferentiated carcinoma composed of irregular, solid sheets of large eosinophilic cells and containing small gland-like spaces.
***Composed of >50% signet ring cells.

the chronology of the clinical, molecular, and pathology hallmarks in the Lynch syndrome.

7. Research funding: Stretching limited funds

Many of Lynch's research efforts in the 1960s and 1970s had been supported by very small grants and personal funds. Although the researchers had applied for significant NIH grant support, they were turned down because reviewers did not believe that genetics was the primary cause for cancer in these families. A common theme that accompanied the "pink slips" explaining the basis for rejection of the grants dealing with Lynch syndrome was the admonition to search for environmental carcinogens, particularly pesticides and herbicides, given the heavy farming background of many of the families.

The research team purchased a recreational vehicle (RV) which they had customized to contain an interview and examining room, and a small laboratory that could be used to prepare blood samples for shipment to collaborators for a variety of studies. This RV cut down significantly on travel costs for the Lynch research team. They took this vehicle to geographic areas of the country where large numbers of family members resided and would educate them as a group about the hereditary disease in their family.

For some of the FISs, particularly when visiting very large families, the hospital outpatient area or a physician's office was also utilized for educational sessions as well as for obtaining rectal mucosal research biopsy specimens for studies of tritiated thymidine labeling of colonic crypts [23]. In some cases the RV was parked at a family member's home, with relatives coming from different parts of the United States. These settings provided an excellent opportunity to get to know the family and meet with them informally. Rapport with the families was significantly enhanced during these sessions. These FISs also appeared to foster a significant group therapy psychological benefit.

Although the RV is long gone, over 150 FISs have been held for hereditary cancer families by Lynch throughout the United States and in many other areas of the world [12].

8. Diagnosis of lynch syndrome

Due to the lack of phenotypic stigmata that might aid in the diagnosis of the Lynch syndrome (Muir-Torre
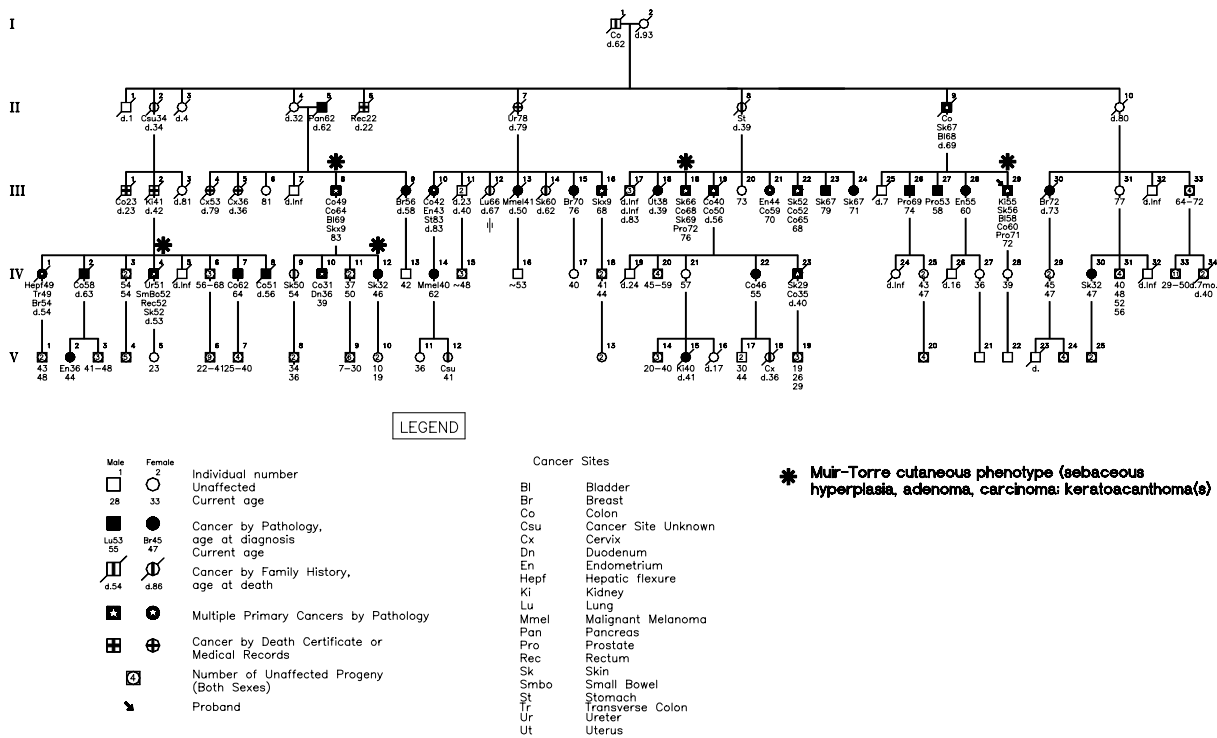
Fig. 2. Pedigree of Muir-Torre family with *MSH2* mutation. (Reprinted with permission from Lynch et al. *Br J Dermatol* 1985;113:295-301.).

syndrome, discussed below, excepted), several sets of diagnostic guidelines have been created. These include the original Amsterdam criteria [24], the less stringent Amsterdam II criteria,25 or the more recently described Bethesda criteria26 as seen in Table 2.

An alternative to these guidelines is "pattern recognition" which involves careful scrutiny of the phenotypic cancer features expressed in the family. This is important when dealing with such factors as small families or reduced gene penetrance of the deleterious MMR mutation. Therein, the presence of the following cardinal features should be considered for a Lynch syndrome diagnosis: (1) autosomal dominant inheritance pattern, as mentioned [15]; (2) gene penetrance for CRC of ≈85–90% [11]; (3) gene carriers develop CRC at an early age (≈45 years) [15]; (4) most (≈70%) of the CRCs are proximal to the splenic flexure [22]; (5) multiple CRCs, both synchronous and metachronous, are common [27,28]; (6) the prognosis is better than that for comparably stage-matched sporadic CRC [29,30]; (7) the pathology features [31] of CRC are often distinguishable (but not pathognomic) and include poor differentiation, increased signet cells, medullary features, peritumoral lymphocytic infiltration, Crohn's-like reaction, and tumor infiltrating lymphocytes (TILs) admixed with tumor cells [32]; (8) there is an increased

risk for malignancy at several extracolonic sites, particularly the endometrium, ovary, stomach, small bowel, hepatobiliary tract, pancreas, ureter, renal pelvis, and brain [13]. Breast cancer excess may be present in some HNPCC families [33]. In Warthin's Family G, gastric cancer was exceedingly common prior to 1900, although it declined in this family in subsequent generations, paralleling its decline in the general population [16]. However, gastric cancer is still prevalent in Lynch syndrome families in Japan and Korea [34]. In addition, accelerated carcinogenesis of CRC, discussed subsequently, occurs in HNPCC [35–37].

9. Muir-torre syndrome (MTS)

In 1981, Lynch et al. [38] reported the first observation of the cutaneous features of MTS in the Lynch syndrome. This phenotype comprised multiple cutaneous sebaceous adenomas, sebaceous carcinomas, multiple keratoacanthomas, and multiple visceral cancers. Several papers [39–42] have elucidated the clinical and molecular genetic features of MTS. Data suggest that the identification of these MTS cutaneous features in a patient merit a detailed family history in the search for evidence of the Lynch syndrome. Indeed, patients with

these stigmata merit germline testing, particularly for evidence of the *MLH2* germline mutation. An extended Lynch syndrome family with MTS that also is known to carry an *MLH2* mutation is shown in Fig. 2.

10. History of lynch syndrome pathology features

Jass [37] elucidated the nature of the pathology of precursor lesions in HNPCC when he postulated the "aggressive adenoma" theory, i.e., adenomas in HNPCC patients form earlier but about as often as in the general population. However, once formed, these colonic adenomas progress to carcinoma more quickly and/or more often than their sporadic counterparts. This finding is consistent with accelerated carcinogenesis in HNPCC, wherein a tiny colonic adenoma may emerge into a carcinoma within two to three years, as opposed to this same process occurring in the general population, which is believed to take eight to ten years [31,35,36,43,44]. Strong clinical evidence in support of this phenomenon comes from a Finnish study showing a marked decrease in colon cancer incidence for HNPCC patients who have regular colonoscopic surveillance with removal of adenomas [45]. Because of accelerated carcinogenesis, proximal colonic predilection, and early age of CRC onset in the Lynch syndrome, we strongly recommend that annual full colonoscopy be initiated at age 25 (Fig. 3).

Distinctive pathology features may be present in Lynch syndrome tumors. The CRCs of Lynch syndrome tend to have a solid growth pattern that accounts for the high frequency of poorly differentiated carcinomas in a Lynch syndrome series [31]. However, these tumors do not behave as aggressively as their failure to form tubules might suggest [46]. Smyrk [47] has pointed out a resemblance to the "undifferentiated carcinoma" described by Gibbs [48] and the "medullary carcinoma" described by Jessurun [49], both of which are reported in small case series to have a better prognosis than typical colon cancer. Similar histological features characterize the 15% of sporadic colon cancers which demonstrate microsatellite instability (MSI+), a characteristic molecular change observed in tumors that lack mutation MMR activity [50]. The Mayo Clinic group has reported that this special histology, which they refer to as "solid-cribiform growth," has a positive predictive value of 53% for MSI+ status [51].

Smyrk's second observation was that the host lymphoid response known as the "Crohn's-like reaction" is more common in HNPCC than in sporadic cancers [52].

This finding has not been consistently true in all series [31], but a similar tendency to form lymphoid aggregates around the tumor appeared to be a feature of sporadic MSI+ colon cancers as well [50].

In the general population, a Crohn's-like reaction is associated with improved prognosis [53] raising the possibility that this phenomenon accounts for the more favorable prognosis observed in HNPCC [30]. Indeed, although MSI-high (MSI-H) CRCs are often diagnosed at a significantly greater depth of tumor invasion, they appear to have a significantly lower overall pathological stage than cancers with microsatellite stability (MSS) [45,54].

Multiple observational studies have shown a survival advantage for HNPCC colon cancer patients when compared to sporadic colon cancer patients [30]. Gryfe et al. demonstrated that HNPCC patients have improved five-year survival (76% vs. 54%) from CRC on a stage for stage basis compared to people with sporadic tumors [54]. Similarly, the overall ten-year survival rates in affected family members is better than that seen in sporadic CRC (68% vs. 48%) [55].

11. Molecular genetics

The molecular genetic era for HNPCC began when Peltomäki et al. [56] through linkage analysis, identified a locus on chromosome 2p as a site for a gene predisposing to HNPCC. Shortly thereafter, a second locus believed to be etiologic for HNPCC was identified on chromosome 3p by Lindblom in Sweden [57]. At this time it was also demonstrated that the tumors occurring in HNPCC patients had a characteristic molecular change called replication error phenotype (RER), which is now called microsatellite instability (MSI) [58–61]. The subsequent recognition that MSI is the consequence of defective DNA replication error repair, or "DNA proofreading," was contributory to the discovery at the 2p and 3p loci of genes for HNPCC, *MSH2* and *MLH1*, which encode proteins involved in the identification and repair of DNA mismatch errors [62–65]. The DNA MMR system performs a proofreading, or "housekeeping," function. Cells lacking effective DNA MMR accumulate mutations at a very high rate [11].

The identification of germline mutations in *hMLH1* and *hMSH2* was quickly followed by the discovery that other genes that encode for members of the MMR complex are mutated in some HNPCC families, establishing significant locus heterogeneity for HNPCC. The genes
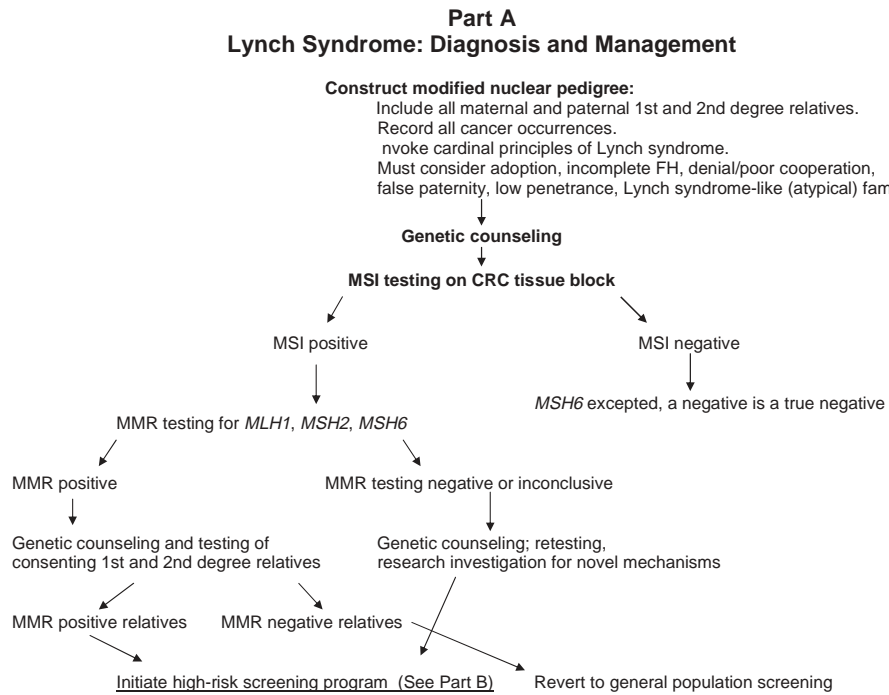
Part A
Lynch Syndrome: Diagnosis and Management

Construct modified nuclear pedigree:
Include all maternal and paternal 1st and 2nd degree relatives.
Record all cancer occurrences.
nvoke cardinal principles of Lynch syndrome.
Must consider adoption, incomplete FH, denial/poor cooperation,
false paternity, low penetrance, Lynch syndrome-like (atypical) family.

↓

Genetic counseling

↓

MSI testing on CRC tissue block

MSI positive   MSI negative

MSH6 excepted, a negative is a true negative

MMR testing for *MLH1, MSH2, MSH6*

MMR positive   MMR testing negative or inconclusive

Genetic counseling and testing of   Genetic counseling; retesting,
consenting 1st and 2nd degree relatives   research investigation for novel mechanisms

MMR positive relatives   MMR negative relatives

Initiate high-risk screening program (See Part B)   Revert to general population screening

Fig. 3a. Algorithm showing proposed diagnosis and management procedures for Lynch syndrome families.

identified to date to cause HNPCC include *hMLH1, hMSH2, hMSH6, hPMS1, hPMS2,* and *hMLH3* [56,57, 62–70].

Ideally, a presumptive hereditary cancer syndrome diagnosis can be confirmed by molecular genetic testing of an affected individual in disorders where deleterious germline mutations have been identified. In the case of the Lynch syndrome, the sine qua non for its diagnosis, namely identification of mismatch repair (MMR) germline mutations, can be established in only about half of clinically diagnosed families. Specifically, Lynch syndrome, as mentioned, is associated with germline mutations in, or the malfunctioning of, post-replicative MMR genes. Two genes, *MLH1* and *MSH2*, account for almost 90% of identified MMR mutations, while MSH6 will account for about 10%. Also seen are mutations in *PMS2, MLH3,* and *EXO1* [71–73].

Why is it that only about half of classical Lynch syndrome families are shown to harbor a MMR mutation or some FAP families lack an identified *APC* mutation? Renkonen et al. [74] in noting that Lynch syndrome as well as FAP families may fail to show any structural change in the presently known susceptibility genes, suggest that either these genes have alterations that escape detection by conventional techniques, or other, as yet unknown, susceptibility genes are involved. These

authors suggest that significant proportions of families presumed to be mutation-negative (up to 11/26 families, or 42%, for HNPCC; up to 4/16 families, or 25%, for FAP) harbor "hidden" alterations in known predisposition genes. They conclude that, "... Evidence of such changes may be obtained by expression-based methods."

These discoveries were made by a variety of talented investigators, including prominently the laboratories of Drs. Bert Vogelstein, Albert de la Chapelle, Richard Kolodner, Päivi Peltomäki, Annika Lindblom, Richard Fishel and Riccardo Fodde.

12. A clinical-molecular genetic model

For diagnostic purposes it is usually sufficient to consider *MLH1* and *MSH2*, and test other genes, particularly *MSH6*, only if mutations are not found in these two. Identification of the culprit predisposing germline mutation in a Lynch syndrome family will determine who should participate in highly targeted cancer surveillance and management programs and, alternatively, those who test negative in a setting where a known mutation has been verified should then follow general population screening guidelines (see Fig. 3).

Part B
Screening and management melded to cardinal features of Lynch Syndrome

Cardinal Features of Lynch Syndrome	Screening/Management
Proximal colonic predilection	Colonoscopy
Early age of onset	Initiate at age 25
Accelerated carcinogenesis	Repeat colonoscopy annually
Predisposition to synchronous and metachronous CRCs	If CRC, subtotal colectomy
Extracolonic cancers: Most Common:	
Endometrial	Endometrial aspiration semi-annually, Transvaginal US
Ovary	Transvaginal US, Doppler color blood flow imagery, CA-125 and repeat annually
Stomach	pper endoscopy, particularly in Orient (Japan, Korea) or families with gastric cancer, repeat annually
Hepatobiliary, small bowel, pancreas	No practical screening with acceptable sensitivity/specificity
Upper uroepithelial tract (ureter, and/or renal pelvis)	Urine cytology, US, positive FH of lesions, families with MTS
Brain	Positive FH, but no known screening efficacy
Sebaceous adenomas, sebaceous carcinomas, multiple keratoacanthomas (MTS)	Cutaneous beacon to screen family for Lynch syndrome tumors, coupled with meticulous cutaneous examinations
Distinguishing pathology features: Poorly differentiated, mucinous features with signet cell excess, increased diploidy, tumor infiltrating lymphocytes, lymphocytic infiltration at periphery, Crohn's-like reaction, increased diploidy	Useful for diagnosis
MMR mutations Most common: *MLH1*, *MSH2*, *MSH6*	Enable certainty in diagnosis
Survival advantage	Evidence which may be useful for genetic counseling

————————

Abbreviations:
CRC: colorectal cancer
FH: family history
MMR: mismatch repair
MSI: microsatellite instability
MTS: Muir-Torre syndrome
US: ultrasound

Fig. 3b. Algorithm showing proposed diagnosis and management procedures for Lynch syndrome families.

13. Microsatellite instability (MSI)

The mentioned new concept of microsatellite instability (MSI) has further provided powerful clues to CRC's carcinogenic pathways and therein it may become a diagnostic aid in the Lynch syndrome and perhaps other disorders yet to be described. For example, Lindblom [75] has described the consequences of genomic instability with respect to distinctive mechanisms involved in proximal and distal CRC.

Jass [76] has provided a review of MSI in CRC with particular attention to the significance of MSI-H in so-called "early-onset" of "sporadic" CRC. He reasons that the molecular profile of early-onset MSI-H CRCs resemble that of HNPCC cancers, even in those cases where Amsterdam criteria fail to be met. Therein, Jass suggests that there are five cogent and interrelated reasons for questioning the presumption that early-onset "sporadic" MSI-H CRCs are truly sporadic: "... First is the fact that the incidence of HNPCC peaks at around 45 years. Second is the finding of germline mutations in DNA mismatch repair genes in subjects presenting with early-onset "sporadic" MSI-H colorectal cancer. Third is the evidence that methylation of *hMLH1* in sporadic MSI-H cancer is strongly age-related. Fourth is the fact that methylation of *hMLH1* may occur selectively in HNPCC cancers in subjects who carry a germline mutation in *hMLH1*. Fifth is the finding of HNPCC-type molecular features among early-onset "sporadic" MSI-H colorectal cancers." Jass [76] appropriately concludes that even the absence of a positive family history should not negate the diagnosis of HNPCC in patients with early-onset CRC characterized by MMR deficiency.

14. International collaborative group on HNPCC (ICG-HNPCC)

In 1989, the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (ICG-HNPCC) was formed to encourage collaborative research studies. In 1991, this group published the rather stringent Amsterdam criteria for HNPCC, which focused exclusively on CRC [24]. These were followed in 1999 by the Amsterdam criteria II [25], which take into account extracolonic cancers that are integral to Lynch syndrome. The international collaboration activities through the ICG-HNPCC have also contributed to the development of a database of identified Lynch syndrome mutations [77], which can be found on the organization's website: http://www.nfdht.nl/. The Bethesda Guidelines followed and were developed to decrease the stringency of the Amsterdam Criteria I and II [26], and make them more useful to clinicians. The guidelines are reviewed in Table 2.

15. International events and the Lynch syndrome

A series of international studies had documented the existence of "cancer families" in countries around the world, including England [78], New Zealand [79], the Netherlands [80], Italy [81], Israel [82], Finland [83], and Uruguay [84]. The Finnish group, by virtue of its access to a population-based cancer registry, was able to demonstrate that the Lynch syndrome was not rare in that country [85]. During this phase of international recognition, the term hereditary nonpolyposis colorectal cancer (HNPCC) came into use. With increased acknowledgment of the importance of extracolonic cancers in the syndrome, coupled with the fact that colonic adenomas do occur in HNPCC, the eponym Lynch syndrome has now become the more accepted terminology [86].

16. Lynch syndrome in South America

Research on Lynch syndrome is ongoing in South America (Uruguay, Argentina, Brazil, and Colombia). These initial investigations began in Montevideo, Uruguay, with a Lynch syndrome family [84,87]. This Uruguayan family was first described in 1977 by Sarroca [84]. Beginning in 1995 [87], the colorectal cancer genetics research team from Creighton University made a series of visits to Uruguay during which time the studies of this original family were extended. Subsequently, the *MLH1* germline mutation was found in the family, segregating in accord with the expected autosomal dominant mode of genetic transmission [87]. Recommendations for surveillance and management were provided. Attention given to this extended family has had a positive impact on the physician community in Uruguay, leading to the identification of additional HNPCC families. Three novel germline mutations which predispose to Lynch syndrome II have been identified in the initial three extended Lynch syndrome kindreds studied in Uruguay [88].

During visits to Uruguay by the Lynch team, techniques for conducting family studies were demonstrated, inclusive of the FIS [12]. Several colorectal surgeons from Argentina and Brazil also attended. These studies have since led to the identification of a large number of Lynch syndrome kindreds throughout these South American countries. These efforts were then extended to studies of the Lynch syndrome in Argentina [89] and Colombia, again through an invitation extended to us by colorectal surgeons in those countries.

17. Finnish HNPCC founder mutation experience

Precedent for the phenomenon of a founder effect in HNPCC originated with de la Chapelle and Wright [90], who evaluated two founder mutations in the *MLH1* gene in Finland. These mutations accounted for approximately half of all HNPCC families in that country [91,92]. These authors found that extensive haplotype sharing, "... over a genomic region as large as 18 cm indicated a relatively recent founding of the more prevalent mutation. ... [wherein] the 'age' of this mutation in most of the 19 kindreds studied could be estimated at 16–43 generations in keeping with historical records and compatible with a founding in a regional subisolate in new Finland in the early 1500s [91, 93]."

Potential cancer control implications from this research abound. These dominant inherited founder mutations spread and their incidence was found to be so high in specific geographic regions of Finland that it constituted a unique public health problem. As these mutations became more readily understood and characterized, efficient screening for them at the population level became possible. Importantly, recognition of these mutations in a patient and his/her family could then signify individuals who could potentially benefit from highly-targeted educational and cancer screening programs [94]. Major public health implications for the diagnosis and, ultimately, prevention of cancer in HNPCC among founder mutation carriers require serious attention by clinical investigators and practicing clinicians [11].

18. Lynch syndrome in the Navajo

The Navajo are a subgroup of the Athabascan linguistic group, who migrated to the southwestern United States from eastern Alaska and Canada about 1000–1200 A.D. [95]. It is estimated that approximately 150,000 Navajo reside in New Mexico and Arizona [95].

In 1983, Thomas Drouhard, M.D., a general surgeon at the Public Health Service Hospital in Tuba City, Arizona, referred a Navajo family to Creighton University (Dr. Lynch). The proband had been diagnosed with ovarian carcinoma and a year later with carcinoma of the cecum. Dr. Drouhard had treated other family members who showed a marked excess of CRC in the absence of multiple colonic adenomas. The pedigree was consonant with Lynch syndrome [15,96] (Fig. 4).

The excess CRC occurrences in this family was especially interesting because of the known paucity of CRC in the Navajo when compared to the general population [97,98].

In collaboration with Dr. Drouhard and his Navajo paramedics, Lynch and his cancer genetics research team visited Tuba City, Arizona, on several occasions to provide information to the family about the syndrome and to obtain blood specimens, skin biopsies, and colonic mucosal biopsies for basic medical genetic research studies of this family. A mutation of a mutL homolog-*MLH1* germline mutation was identified by molecular genetic colleagues in 1994 [99]. In the following year, we provided DNA-based genetic counseling to 23 family members, 7 of whom were positive for the *MLH1* mutation [96]. Their reactions ranged from full acceptance of the genetic implications to certain more traditional Navajo concepts such as, "Our family has been cursed."

Following the identification of the *MLH1* germline mutation in this original Navajo family, the investigators began seeking other nuclear families throughout the Navajo reservation in southeast Arizona and northern New Mexico. The same *MLH1* mutation was found in four presumably unrelated families. These findings were strongly suggestive of a founder effect with the *MLH1* gene. Specifically, sequence analysis of the *MLH1* gene revealed a 4-base-pair deletion beginning at the first nucleotide of codon 727 which predicts a frameshift and a substitution of new amino acids to the COOH-terminus of the protein [65].

Many of the Navajo live on reservation lands, where they have undergone relatively little genetic mixing with other racial groups. This racial homogeneity and the generally common environmental exposures of reservation life make it likely that the evaluation of cancer family history and lifestyle among the Navajo could elicit important epidemiologic clues about host and environmental interaction in cancer etiology. Prospective studies using the *MLH1* founder mutation identified in these Navajo families could facilitate such genetic-epidemiologic research [99]. This would allow the identification of environmental exposures in concert with knowledge of who is versus who is not inordinately predisposed to cancer.

19. North American founder mutation

Lynch et al. [100] have described another example of the founder mutation phenomenon. This involves a mu-
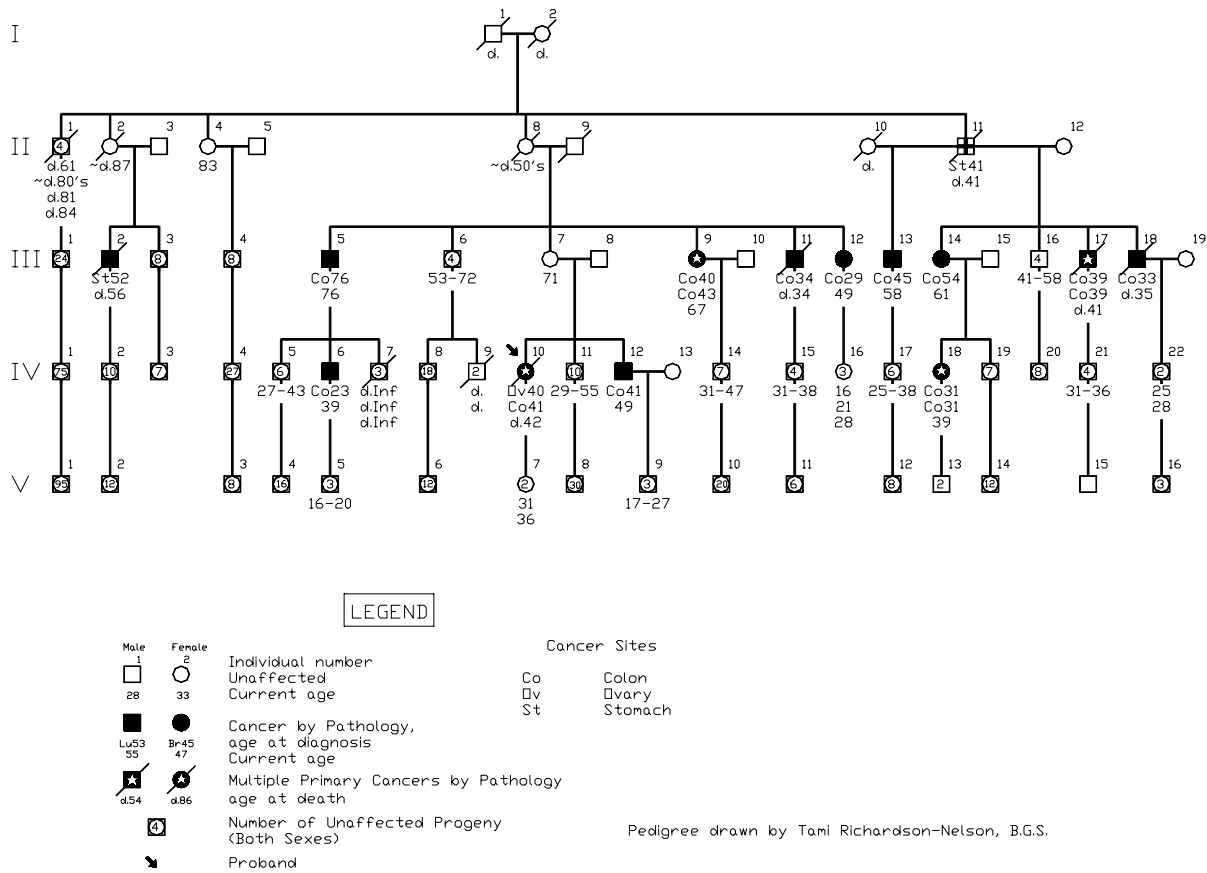
Fig. 4. Pedigree of Navajo family with *MLH1* mutation. (Adapted with permission from Lynch et al. *Cancer* 1996;77:30–35.) Lynch and Lynch/Page 12.

tation, namely *MLH2* del [1–6], in nine families which have been tracked from their "founder" in Germany in the eighteenth century through their migrations to and within the United States until the present day [101].

Founder mutation studies have many advantages compared to genetic testing in unrelated populations, including more efficient identification of relatives who are at increased hereditary cancer risk and who thereby can benefit from genetic counseling in concert with highly targeted surveillance and management.

20. Discussion

We have attempted to describe many of the important historical landmarks of the Lynch syndrome (HNPCC), the most commonly occurring hereditary syndrome that predisposes to CRC [11]. HNPCC is complex, and thereby, during the past decade, diagnostic guidelines for HNPCC have been modified significantly [24–26], as have advances in surveillance, management [11,35,

94], and molecular genetic testing [7,71]. Needed is greater understanding of the ethical and malpractice issues that impact on these concerns [102], and of the barriers to its diagnosis, management, and compliance of at-risk patients.

The original definitions of HNPCC based upon clinical and pedigree criteria such as the more stringent Amsterdam criteria [24] or the less stringent Amsterdam II criteria [25] are valid today. However, in many situations the occurrence of HNPCC-associated cancers, especially in small families, cancer of markedly early onset, or the pattern of multiple cancers in any single individual, should alert the clinician to the possibility of HNPCC.

21. What the physician needs to know: CRC as a model

Calvert and Frucht [103] stress the need for physicians to understand the molecular causes of CRC, with

particular recognition of the variation in cancer phenotypes, in the quest for improved cancer screening and appropriate genetic testing in the interest of prevention.

Soravia et al. [104] note how the causative genes for the several hereditary CRC-prone syndromes have become widely used for confirming their clinical diagnosis. Thus, genetic testing may provide indications for targeted surveillance of at-risk family members and, conversely, may allow those family members who are not harbingers of the deleterious gene present in the family to avoid this intensive surveillance and management. These issues are particularly important in the management of the Lynch syndrome because, with the single exception of the cutaneous stigmata of the MTS, there are no obvious phenotypic physical stigmata that will aid in its diagnosis. Therefore, the diagnostician must continue to rely heavily upon the family history, cancer phenotype, pathology findings and, when available, the presence of cancer-causing Lynch syndrome MMR mutations (Fig. 3).

Chemoprevention and chemotherapy in the management of the Lynch syndrome remain challenging areas of concern, with new knowledge having the potential to impact every area of cancer management and treatment. For example, the findings of Ribic et al. [60] suggest that while adjuvant therapy with 5-fluorouracil improved survival among CRC patients (stage II and stage III) with MSI-stable and MSI-low tumors, it had no benefit for those with MSI-high tumors.

22. Cancer control in lynch syndrome

Järvinen and colleagues [94] demonstrated the benefit of colonoscopic screening in HNPCC through a controlled clinical trial extending over 15 years. The incidence of CRC was compared in two cohorts of at-risk members of 22 HNPCC families. CRC developed in eight screened subjects (6%), compared with 19 unscreened controls (16%; $p = 0.014$). The CRC rate was reduced by 62% in those who were screened. All CRCs in the screened group were local, causing no deaths, compared with nine deaths caused by CRC in the controls. It was concluded that CRC screening at three-year intervals more than cuts in half the risk of CRC, prevents CRC deaths, and decreases overall mortality by about 65% in HNPCC families. The relatively high incidence of CRC even in the screened subjects (albeit without deaths) in our opinion argues for shorter screening intervals, e.g., one year. For example, Vasen and colleagues [105] discovered five interval cancers in

HNPCC patients within 3-1/2 years following a normal colonoscopy.

In reviewing this subject, Church [106] suggests that interval CRCs develop from normal epithelium within three years and/or from adenomas that were missed. It is also important to realize that colonoscopy "miss" rates are as high as 29% for polyps <5 mm in diameter [107]. Patients should, therefore, be advised that colonoscopy, while not a perfect screening procedure, is, nevertheless, highly effective [94]. The option of prophylactic colectomy should be discussed [108,109], particularly in non-compliant patients, as described below.

23. Prophylactic colectomy

Subtotal colectomy as a prophylactic measure among HNPCC patients remains controversial. However, in special circumstances, patients who carry germline MMR cancer-causing mutations should be offered this option as an alternative to lifetime colonoscopic surveillance. Genetic counseling, coupled with a second surgical opinion, must be provided so that patients can be in the best possible position to evaluate the various available surgical management strategies. Church109 and Lynch108 have suggested that prophylactic surgery should be an option for those patients likely to show reduced compliance for colonoscopy.

Some authors [110–113] have found the 70% to 80% lifetime risk of developing CRC to be a compelling rationale for prophylactic surgery, while others disagree [114–117]. Clearly, prophylactic colectomy should be offered only in selected situations, such as in a mutation carrier who completely refuses colonoscopy surveillance. The patient must understand that after surgery, surveillance of the remaining rectal segment will need to be performed annually with sigmoidoscopy [110]. We argue that prophylactic colectomy in Lynch syndrome, while an unsettled problem begging for a scientific evidence-based answer, must be individualized in accordance with the patient's best posits for cancer control.

Syngal and colleagues [115] examined the life expectancy and quality-adjusted life expectancy benefits resulting from endoscopic surveillance and prophylactic colectomy among carriers of germline mutations for HNPCC. Both risk-reduction programs showed large gains in life expectancy for mutation carriers, with benefits of 13.5 years for surveillance and 15.6 years for prophylactic proctocolectomy at 25 years of age, compared with no intervention. The benefits of prophylactic colectomy decreased with increasing age.

24. Prophylactic hysterectomy and oophorectomy in Lynch syndrome

Women at risk for the Lynch syndrome should have annual screening for endometrial and ovarian cancer beginning at age 30 to 35 years. Endometrial aspiration coupled with transvaginal ultrasound is advised for screening. CA 125 testing should be performed semi-annually for ovarian cancer. Women must be advised of the marked limitations in ovarian cancer screening. Prophylactic hysterectomy and oophorectomy can be considered when childbearing is completed.

25. Future projections

Finally, there remain countless areas in the etiology, pathogenesis, and control of HNPCC that will require continued intensive research. Some of the questions to be answered are: 1) What is the complete tumor complement of HNPCC? 2) What are the chemotherapy and chemoprevention implications of this disease? 3) Can we improve surveillance/management strategies? 4) Can we achieve molecular-based chemoprevention? 5) What are the genotypic and phenotypic heterogeneity implications of Lynch syndrome? 6) What are the differential diagnostic implications?

Our efforts and those of colleagues throughout the world have only grazed the tip of the iceberg in terms of the etiology, pathogenesis, surveillance, and management of Lynch syndrome. What is known in high relief is that the knowledge accrued to date, when translated clinically, can save lives!

Acknowledgements

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