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An Update of HNPCC (Lynch Syndrome)

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ABSTRACT: *Genetic epidemiology studies of colorectal cancer (CRC) can identify persons who are at inordinately high risk and who thereby might benefit from targeted early detection and primary prevention programs, inclusive of prophylactic surgery in selected cases. The discipline of molecular genetics has identified germline mutations that include APC in familial adenomatous polyposis (FAP) and mutator genes, namely MSH2, MLH1, PMS1, and PMS2 in hereditary nonpolyposis colorectal cancer (HNPCC). These discoveries have significantly enhanced our ability to identify individuals whose cancer destiny can literally be determined at birth. This review updates HNPCC's differential diagnosis, heterogeneity, tumor spectrum, newly found evidence of accelerated colonic adenoma to CRC, survival advantage, and currently available surveillance and management programs. Emphasis has been on how knowledge of the genetics and natural history of HNPCC can be used effectively to promote early diagnosis or prevention of cancer.* © Elsevier Science Inc., 1997

INTRODUCTION

Approximately 133,500 new cases of CRC will be diagnosed in 1996, and 54,900 will die of this disease (27,400 males and 27,500 females) [1]. Despite progress in chemotherapy, radiation therapy, and surgery, there has been little improvement in the survival of patients with CRC during the past several decades. This failure underscores the need for research into the epidemiology and etiology of CRC, in the interest of identifying high-risk groups who might benefit from early detection and prevention. Clinical and molecular genetics have been at the forefront of this effort, enhancing our ability to identify individuals with increased lifetime risk for CRC.

This review is concerned with the two principal hereditary forms of CRC, FAP, and HNPCC. We will provide a historical background of these diseases, and will describe their natural history, molecular genetics, and differential diagnosis (Table 1), all in concert with the genetic heterogeneity of hereditary CRC (Fig. 1). Emphasis will be on how the knowledge of genetics can be employed effectively in clinical practice to maximize early diagnosis, direct DNA-based genetic counseling, and prevent malignancy through prophylactic surgery.

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FAP

History of FAP

Bulow [2] has traced the history of FAP to a report in 1861 by Luschka [3] that "may have been FAP." The first definite example of a patient with multiple colonic polyposis was published in 1881 by Sklifasowski [4]. The first "familial" example was that of a brother and sister with FAP reported by Cripps [5] in 1882. Examples of CRC occurring in patients with FAP were reported by Smith in 1887 [6]. Description of the histologic progression from adenoma to adenocarcinoma was first described by Handford [7]. Lockhart-Mummery [8] was the first to express the idea that the hereditary factor in this disease was not for cancer per se, but for multiple adenomas with a tendency to undergo malignant transformation. Lockhart-Mummery's report was based on findings from three families that formed the original basis for the now-renowned St. Mark's Hospital Polyposis Registry (London, England).

The APC Gene

The discovery of the APC gene was aided immeasurably by the landmark discovery of Herrera [9] who worked with Avery Sandberg and others to identify a large deletion on chromosome 5q in a 42-year-old patient with multiple congenital abnormalities and multiple colonic polyps. This discovery provided the first clue about where to direct the search for the gene using linkage analysis. [10–12], leading ultimately to the cloning of the gene [13, 14].

A great deal has been learned about the APC gene and the function of its gene product. One interesting finding is the relationship between the location of mutations in the gene and the phenotypic expression of FAP. For example, an attenuated variant of FAP [15] with fewer adenomas

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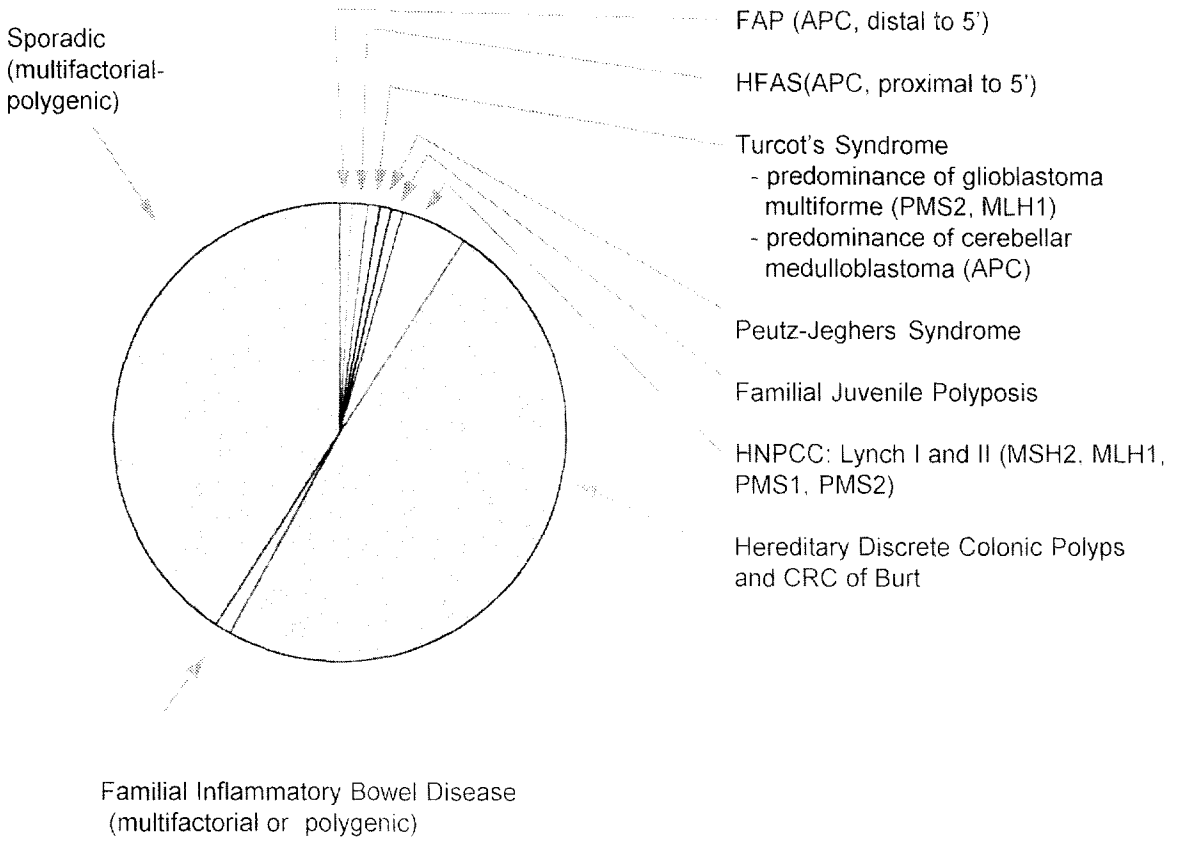


Figure 1 Pie diagram showing the extant heterogeneity in hereditary colorectal cancer. (Reproduced with permission. Lynch HT, Smyrk T, Jass JR (1995): Sem Surg Oncol 11:406–410).

and lower cancer risk is attributable to a mutation near the proximal (5') end of the gene [16], whereas more distal mutations are associated with the classical form of FAP. In addition, Caspari et al. [17] found a correlation between mutation sites and the physical sign of congenital hypertrophy of retinal pigment epithelium (CHRPE). Individuals with mutations in codons 136–302 do not develop CHRPE, those with mutations in codon 1463–1387 regularly show CHRPE, and mutations in codons 1445–1578 consistently lack CHRPE. Furthermore, individuals with mutations in codons 1445–1578 develop an increased susceptibility to desmoid tumors.

The correlation between genotype and phenotype is not perfect, identical mutations may produce different physical manifestations in different patients. It may be that environmental modifiers affect phenotypic expression or that other genes interact with the APC gene to affect phenotype. One candidate for a genetic modifier is a locus on chromosome 1 [18].

Need for Cancer Control

Physicians continue to miss high-risk patients, despite the often obvious phenotypic manifestations of FAP, in part because family histories are incomplete. Arvanitis et al. [19] discussed this problem and showed that the majority (59%) of patients with FAP die of metastatic CRC. Clearly, this dismal outcome is intolerable and could be resolved by providing patients who are at increased risk for FAP with regular surveillance and prophylactic colectomy when the polyposis phenotype is identified. However, for cancer control to be effective, patients at increased CRC risk from FAP families must be identified *before* cancer occurs. Herein, compiling a well orchestrated family history is essential to this process, followed by extending this knowledge to the FAP proband's primary and secondary relatives who are at risk.

Table 1 provides a composite of the phenotypes, surveillance, management, genetics, and, when known, location or identification of the culprit genes in the several hereditary CRC syndromes.

HNPCC

History of HNPCC

The historic evolution of HNPCC is outlined in Figure 2 [20–56]. The history of the syndrome now known as HNPCC dates to 1895, when Aldred Warthin, curious about his seamstress' depression, heard that her family history made her feel certain she would one day die of cancer of the female organs or bowels. As predicted, she died of endometrial carcinoma at a young age. This malignancy, gastric carcinoma and CRC, occurred repeatedly in

Table 1 Hereditary colorectal cancer: differential diagnosis, surveillance and management, and germline mutation where known

Hereditary form of colorectal cancer (CRC)	Inheritance pattern/ germline mutation	Polyps	Cancer	Non-cancer features	Screening	Surgical management and/or prophylaxis	Presymptomatic DNA testing	Genetic counseling
Familial adenomatous polyposis (FAP)	AD; APC gene at chromosome 5q, distal to 5[1]	Adenomatous, often start in distal colon/rectum; usually >100; Adenomas may occur in small bowel; gastric polyps common, usually fundic gland polyps	CRC, average age onset 39; many cases teens and twenties; cancer of small bowel; stomach (particularly in Japan), papillary thyroid cancer, periampullary carcinoma, sarcoma, brain tumor	Gardner's variant-epidermoid cysts of skin, osteomas of mandible, congenital hypertrophy retinal pigment epithelium. Desmoid tumors (intraabdominal) do not metastasize but may kill by direct extension; Desmoids may be initiated by surgery (dissected surfaces); adrenal adenomas	Baseline flexible sigmoidoscopy age 10–12; flexible sigmoidoscopy annually thereafter, for APC germline positive. If at risk but not tested for APC, same strategy. If eventually found to be APC negative, then baseline flexible sigmoidoscopy at age 15–20, if negative, no further screening. Upper endoscopy every 1–3 years starting when colonic poyps first appear	Prophylactic subtotal colectomy with low ileal-rectal anastomosis when phenotype (florid polyposis) identified; may consider rectal mucosectomy with ileal pouch anal anastomosis if too many rectal polyps to manage or if compliance for rectal segment follow-up is poor. Consider Sulindac chemoprevention (while reducing polyps, cancer may still occur).	Test for APC germline mutation as early as ages 10–12.	Initiate preteens, include parents
Attenuated familial adenomatous polyposis coli (AFAP)	AD; APC gene at chromosome 5q, proximal to 5[1]	Ordinary adenomas but also flat adenomas with proximal colonic predominance; may be few (5–10), sometimes >100	CRC with average age onset at 50; occasional periamullary carcinoma	Fundic gland polyps in stomach. Adenomas in duodenum	Colonoscopy, and upper endoscopy, initiate at age 20 and annually for APC germline positive patients or every 2 years if at genetic risk but not tested for APC	Prophylactic subtotal colectomy if too many polyps to manage; consider chemo preventive Sulindac	Test for APC germline mutation at age 20	Initiate at age 20, include parents
Turcot's Syndrome	AD, both FAP and HNPCC variants	Florid colonic adenomas, 5 to >100	CRC and central nervous system, particularly brain tumors	Rare examples of multiple *cafe-au lait* spots and pigmented nevi but not clear if truly integral to the syndrome	Baseline flexible sigmoidoscopy age 10 to 12 and annual flexible sigmoidoscopy thereafter; consider CAT scan or MRI brain	Prophylactic subtotal colectomy if colonic polyps present, as in FAP	Two DNA variants: 1) APC gene with predominance of cerebellar medulloblastoma. 2) hMLH1, or hPMS2, with predominance of glioblastoma multiforme	Initiate age 10–12, include parents
Juvenile polyposis coli	AD	Diffuse hamartomatous, (may have adenomatous component) of colon, but may occur in small bowel and stomach	CRC	Children may manifest diarrhea (may be severe)	Initiate colonoscopy age 10–12	Prophylactic subtotal colectomy when phenotype present with too may polyps to manage	None known	Initiate preteens, include parents
Peutz-Jeghers Syndrome	AD	Peutz-Jeghers polyps (may have adenomatous features) in stomach, small bowel and colon	Stomach, small bowel, colon, sex cord tumors ovary and testes	Mucocutaneous melanin pigmentation	Baseline colonoscopy, and upper endoscopy, initiate age 20, flexible sigmoidoscopy annually thereafter.	Consider prophylactic subtotal colectomy if too many polyps to manage and if mixed adenomatous features	None known	Initiate teens and include parents
Discrete colonic adenomatous polyps and CRC of Burt	AD, may be the same as familial CRC	Occasional (never florid) adenomatous colonic polyps	CRC, average age in accord with population expectations	None known	Initiate baseline flexible sigmoidoscopy at age 40 and thereafter, every 3 years	Standard CRC surgical approach	None known	initate at age 25–30

(*continued*)

Table 1 Continued

Hereditary form of colorectal cancer (CRC)	Inheritance pattern/ germline mutation	Polyps	Cancer	Non-cancer features	Screening	Surgical management and/or prophylaxis	Presymptomatic DNA testing	Genetic counseling
Hereditary non polyposis colorectal cancer (HNPCC)	AD, germline mutations of any of the mismatch repair genes: MSH2 at chromosome 2p; MLH1 at chromosome 3p; PMS1 at chromosome 2q; PMS2 at chromosome 7q	Occasional colonic ademonas which are on average larger, more villous, and at younger age than general population	CRC most common with proximal predominance, an excess of synchronous and metachronous CRC. Others include cancer of the endometrium, ovary, small bowel, stomach, and transitional cell carcinoma of ureter and renal pelvis. Average age of cancer onset is 44; may show rapid progression from adenoma to CRC	Muir-Torre syndrome variant shows cancer features of HNPCC but includes sebaceous adenomas, sebaceous epitheliomas, basal cell epitheliomas with sebaceous differentiation. Meibomian gland carcinomas and sebaceous carcinomas; single or multiple keratoacanthomas	Colonoscopy, initiate age 20–25, annually for germline mutation carriers. Every other year when mutation studies are lacking; Endometrial aspiration biopsy at the same time as colonoscopy	Subtotal colectomy for initial CRC; consider option of prophylactic subtotal colectomy for germline carriers. Consider prophylactic total abdominal hysterectomy and bilateral salpingo oophorectomy for patients with initial CRC who have completed their families	Test for germline mutations no earlier than age 18–20	Initiate at age 18, prior to any consideration for gene testing
Familial CRC	Empirical risk three fold increase for CRC in patients with one or more first degree relatives with CRC; likely multifactorial and/or low penetrant genes	In accord with population expectations	CRC, comparable to age of onset and colonic location for general population	None	Baseline flexible sigmoidoscopy at age 40 and repeat every 3 years; if two first degree relatives affected or one less than age 50 years, risk is 4–6 fold increased and full colonoscopy every 3–5 years indicated.	Standard surgical procedure for CRC	None	Initiate at age 30–35
Familial ulcerative colitis and Crohn's Disease	Unknown; possible AD in some families, polygenic also likely	Pseudopolyps (non-adenomatous)	CRC, lymphoma of GI tract	Arthritis, pyoderma gangrenosum, annular erythemas, and vascular thromboses, sclerosing cholangitis	Colonoscopy, annual in patients with chronic pan colitis of 8 or more years duration; check for high grade dysplasia colonic mucosa	Subtotal colectomy for CRC; consider prophylactic subtotal colectomy for patients with persistent high grade dysplasia of colonic mucosa; proctocolectomy if IBD mandates	None	Initiate at age 18–20

AD, autosomal dominant; CRC, colorectal cancer; and IBD, inflammatory bowel disease.

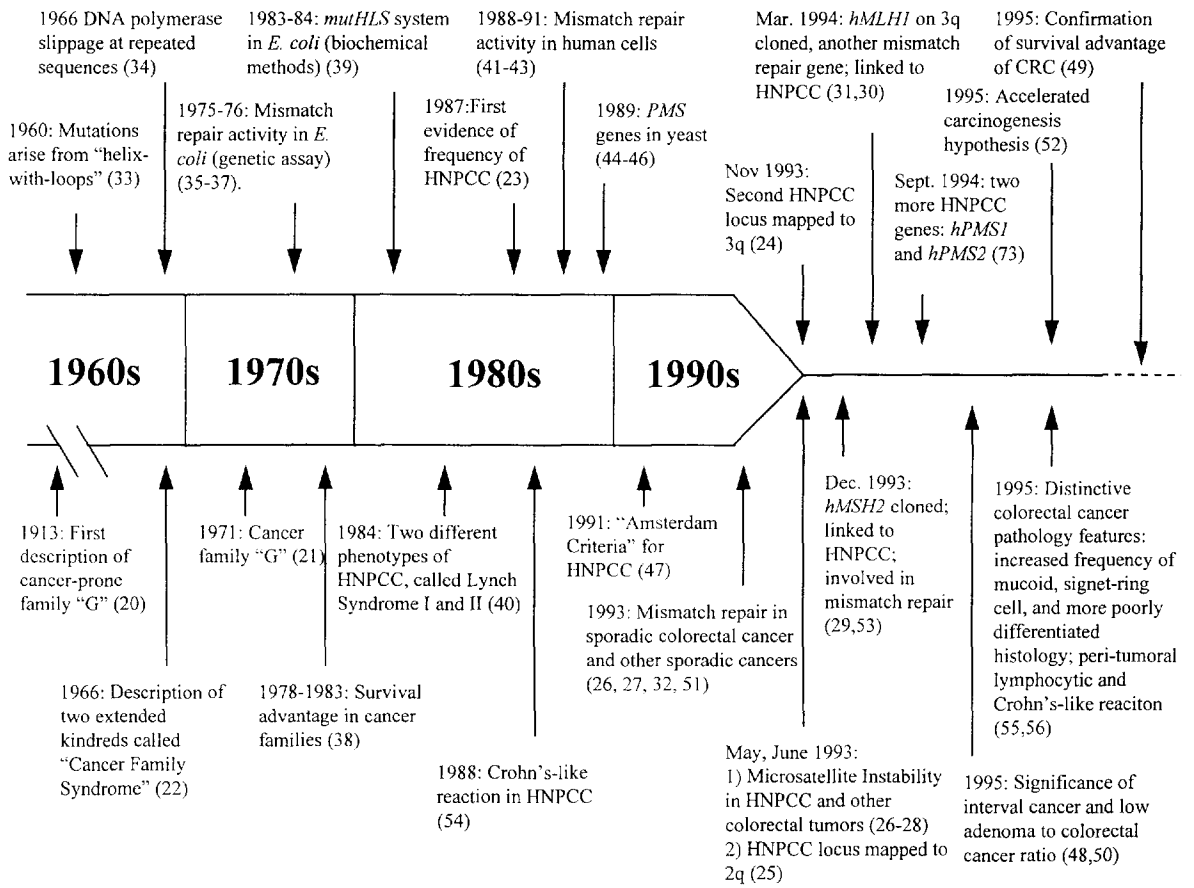


Figure 2 Timeline for clinical, pathology, and molecular genetic studies in hereditary nonpolyposis colorectal cancer. (Modified from G. Marra and C.R. Boland, 1995: J Natl Ca Inst 87:1114–1125; reproduced with permission. Lynch HT, Smyrk T(1996): Cancer 78:1149–1167).

her kindred, Warthin's Family G [20]. Gastric carcinoma was the predominant lesion in Family G in Warthin's initial descriptions of the kindred [57, 58], but in the most recent update, gastric cancer was less common (paralleling its decline in the general population) and CRC was more common [21] .

The significance of Family G's aggregation of colonic, gastric, and endometrial carcinoma was not fully appreciated until two extended kindreds were described under the appellation of Cancer Family Syndrome (CFS) [22]. One of the CFS families (Family N), was ascertained in 1961. The proband had been in delirium tremens. When asked why he drank, he stated that he did so because he was going to die of cancer because ". . . everyone in the family dies of cancer," a sentiment strikingly similar to that of Warthin's seamstress.

A series of international studies documented the existence of cancer families in countries around the world, including England [59], New Zealand [60], the Netherlands [61], Italy [62], Israel [63], and Finland [64]. The Finnish group has access to a population-based cancer registry, and demonstrated that the syndrome was not rare in that country [23]. During this phase of international recognition, the term HNPCC came into use [61].

Clinical Features and Frequency of HNPCC

HNPCC is characterized by early age of cancer onset (≈44), proximal predominance of CRC (≈70% proximal to splenic flecture), multiple synchronous and metachronous CRCs (≈45% within 10 years after incomplete colonic resection), and an excess of certain extra-colonic cancers. Watson and Lynch [65] evaluated extracolonic cancers in the Creighton HNPCC resource and identified endometrial carcinoma as the second most common lesion (after CRC), followed by carcinoma of the ovary, stomach, small bowel, hepatobiliary tract, and transitional cell carcinoma of the ureter and renal pelvis. Of interest, there was a statistically significant deficit of carcinoma of the lung. Carcinoma of the breast showed a statistically nonsignificant deficit, but, when breast cancer was encountered in HNPCC families, it frequently occurred at an early age.

HNPCC is not rare; it probably accounts for at least 5% of all CRCs. All attempts to investigate the frequency of HNPCC are handicapped by the fact that until very recently the diagnosis of HNPCC rested on descriptive criteria. In an effort to standardize reporting of putative HNPCC families, an international panel meeting in Amsterdam put forth a list of criteria (Table 2) that must be satisfied for a diagnosis of HNPCC. The Amsterdam criteria are fairly re-

Table 2 Amsterdam criteria for HNPCC

1) At least three family members with CRC, two of whom are first degree relatives.
2) At least two generations represented.
3) At least one individual less than 50 years old at diagnosis.

strictive; small families are not likely to meet criteria for diagnosis, and extracolonic malignancies, clearly an important component of the syndrome, are not given any diagnostic weight. Estimates of HNPCC's frequency based on the Amsterdam criteria, then, are likely to be low.

Illustrative HNPCC Families

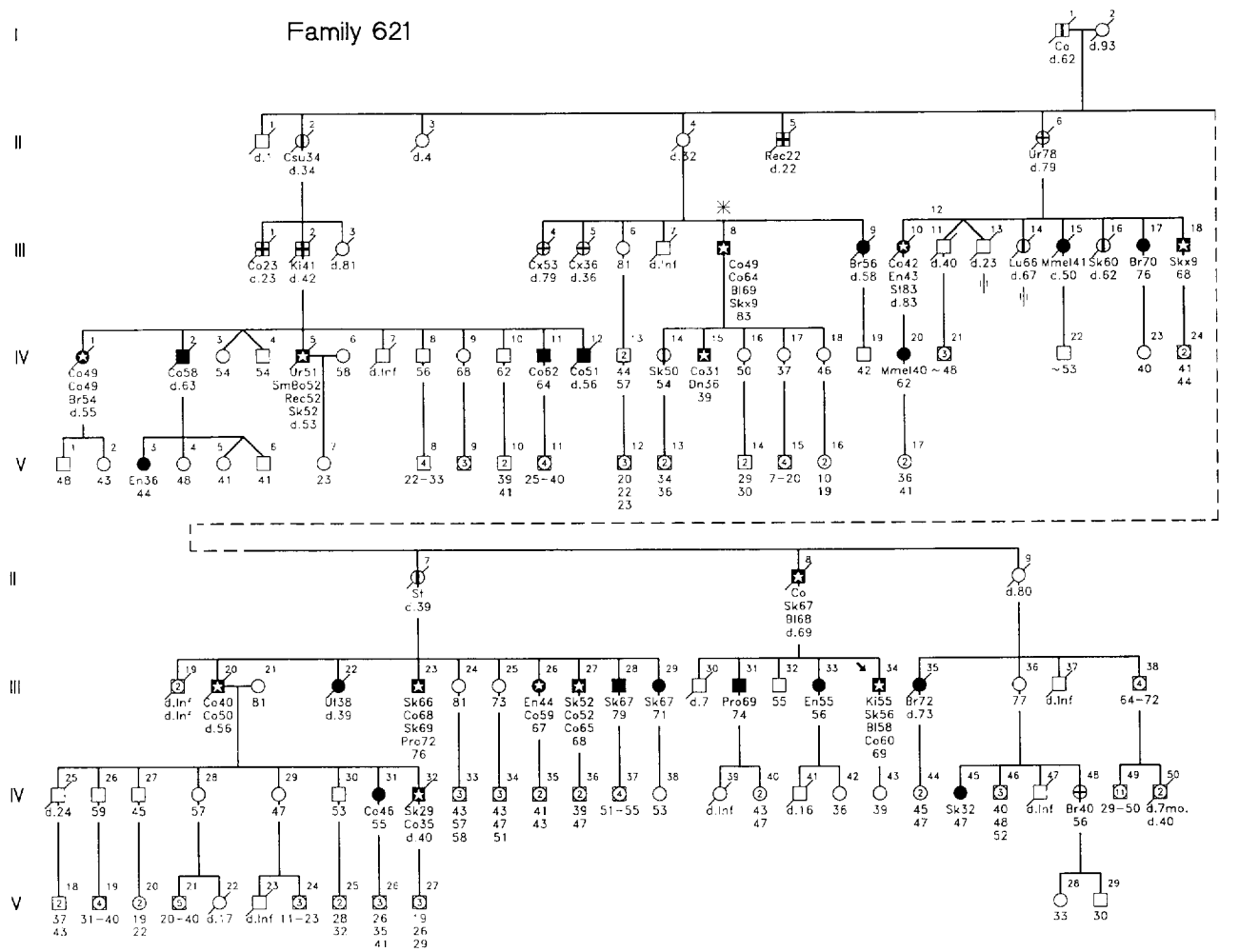
The Creighton University HNPCC registry contains more than 150 extended kindreds. Germline mutations for MSH2 and MLH1 have been identified in 12 of these families. The following five families are representative of this resource and they have been selected to show features that are often classic for the HNPCC syndrome diagnosis.

Family 621 (Figure 3). This exceedingly large kindred shows findings consistent with the Muir-Torre cutaneous stigmata, which is a variant of the Lynch syndrome II. The proband (III-34) has had multiple primary cancers including kidney, urinary bladder, and colon. In addition, he has shown the classic stigmata of Muir-Torre syndrome as characterized by sebaceous adenomas, sebaceous carcinomas, and multiple keratoacanthomas.

There is direct transmission of Lynch syndrome II varieties of visceral cancer through five generations. Muir-Torre cutaneous phenotype occurred in three other family members (III-8, III-29, IV-18). The mutation has not been identified in this family.

Family 7 (Figure 4). This Lynch syndrome II family is of Native American extraction. They reside on their reservation in Arizona. This Navajo family was referred to us by a sur-

Figure 3 Updated pedigree of an HNPCC family also showing Muir-Torre cutaneous phenotype occurring in four patients (III-8, III-29, III-34, and IV-18). (Reproduced with permission. Lynch HT, Fusaro RM, Roberts L, Voorhees GJ, Lynch JF (1985): Br J Derm 113:295–301).



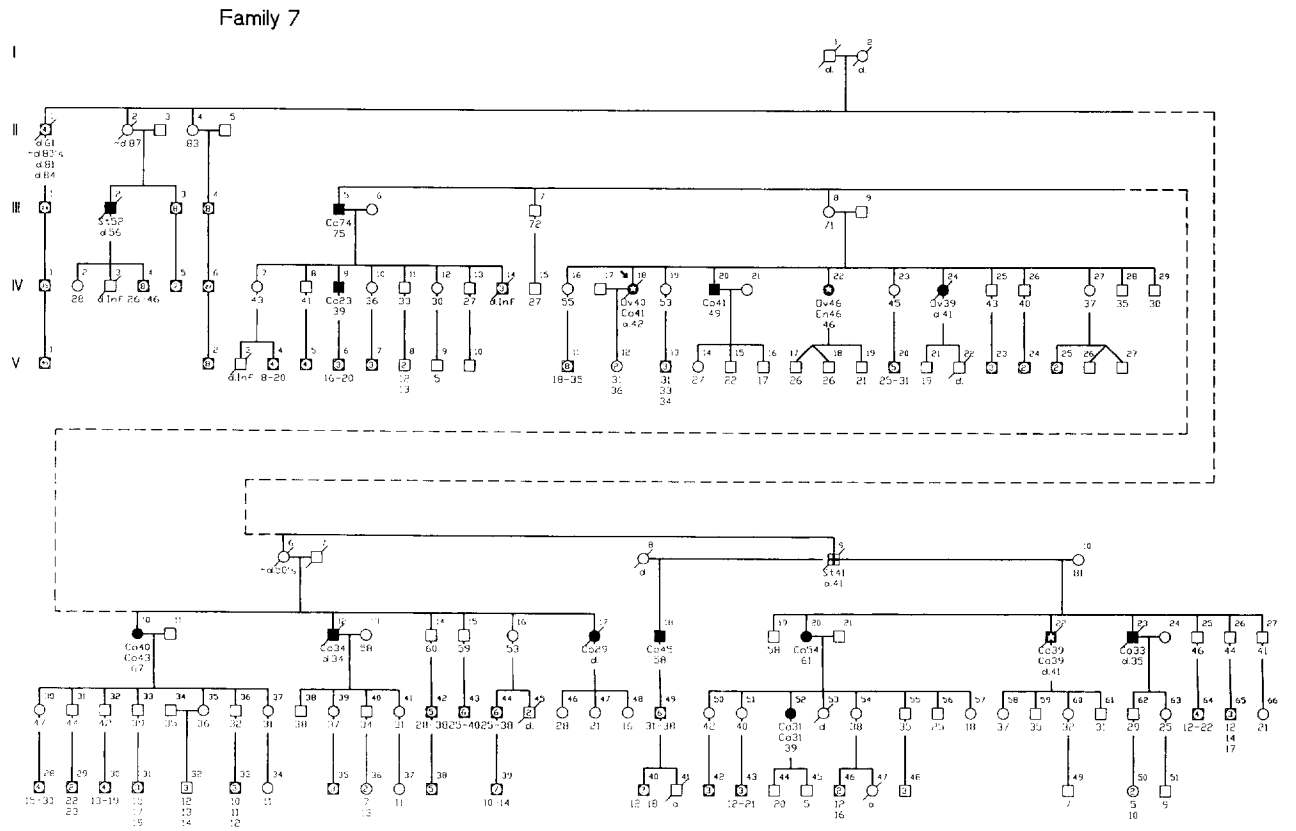


Figure 4 Pedigree of an extended HNPCC family of Navajo extraction with MLH1 germline mutation. Colorectal carcinoma is uncommon in Native Americans. (Reproduced with permission. Lynch HT, Drouhard T, Vasen HFA, Cavalieri J, Lynch JF, Nord S, Smyrk T, Lanspa S, Murphy P, Whelan K, Peters J, de la Chapelle A (1996): Cancer 77:30–35).

geon in 1982 because of an apparent excess of CRC among the first-degree relatives of the proband (IV-18) who presented with carcinoma of the ovary at age 40, and a second primary carcinoma of the colon at age 41. Two of her sisters (IV-22, IV-39) also had ovarian cancer at early ages and a brother had adenocarcinoma of the colon at age 41. DNA was obtained from peripheral blood lymphocytes to test for linkage. Following exclusion of the 2p chromosome locus, MLH1 mutation was identified in two affected individuals from separate branches of the family. Compliance to screening recommendations has been disappointing [66].

Family 3 (Figure 5). This is a typical Lynch syndrome II kindred. The proband (III-1) had cancer of the ascending colon initially and underwent a hemicolectomy instead of subtotal colectomy. She developed endometrial carcinoma at age 51. She then presented with two primary lesions in the transverse colon, one at age 57 and the second at age 63. She then had another cancerous lesion in the descending colon at age 77. Of her six siblings, five have manifested the Lynch syndrome cancers of the colon and endometrium. One member (III-3) had gastric carcinoma, a lesion that occurs infrequently in Lynch syndrome II patients in the United States, but is more common in the Orient.

Family 4 (Figure 6). This kindred shows a proclivity to CRC and has a marked paucity of extracolonic cancers. We believe that this family constitutes the Lynch syndrome I variant. To date, the mutation in this family has not been found.

Family 3208 (Figure 7). This Lynch syndrome II kindred was ascertained initially by a gynecologic oncologist who wondered about the presence of a hereditary cancer syndrome based upon two sisters (IV-9, IV-10) who manifested early onset synchronous carcinomas of the endometrium and ovary. Their mother (III-6) had ovarian carcinoma. The family was referred to us for further study. The maternal lineage did not support a diagnosis of the Lynch syndrome. However, when the paternal lineage was extended it became evident that the tumor pattern was consistent with Lynch syndrome II. Note also the presence of pancreatic cancer in patient III-3 and his daughter, IV-5. Pancreatic cancer has been shown to be an integral lesion in a subset of Lynch syndrome II kindreds. The HNPCC diagnosis in this family would have been missed if the Amsterdam Criteria had been invoked. Figure 8 shows the legend to Figures 3–7.

Molecular Genetics

Minna Nyström-Lahti [67] has traced the history of the molecular genetics of HNPCC. The landmark study by Pel-

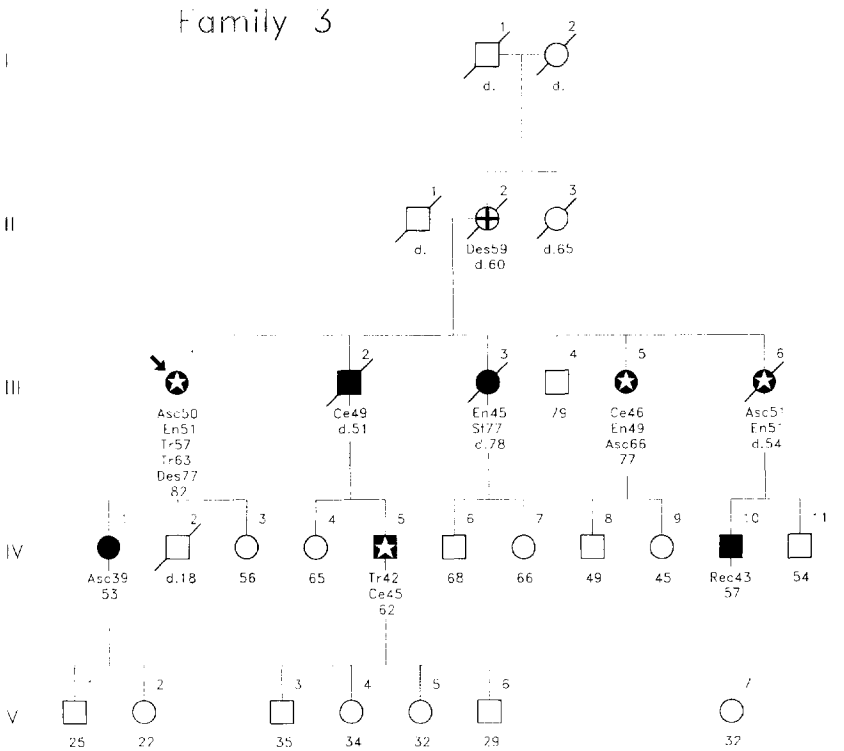


Figure 5 An updated pedigree of one of the first representative HNPCC families. Note the several occurrences of colon cancer and endometrial carcinoma in the same patient. (Reproduced with permission. Lynch HT, Lynch PM, Harris RE (1978): JAMA 240:535–538).

tomaki et al [51] mapped the first HNPCC locus to chromosome 2p15-16 in two large HNPCC families, one from Canada and one from New Zealand. Almost immediately, Lindblom et al [24] found a second HNPCC locus on the short arm of chromosome 3 by linkage analysis in two Swedish families.

Both linkage studies noted above used microsatellite markers to map the HNPCC genes. Microsatellites are repeating sequences of unknown function distributed throughout the genome. The most common repeats are (A)n/(T)n and (CA)n/(GT)n. In addition to providing markers for linkage studies, DNA microsatellites supplied a critical hint about the nature of the genetic defect in HNPCC. Working with sporadic CRC, Ionov et al [26] and Thibodeau et al [27] observed that some tumors featured microsatellite instability; that is, the length of the microsatellites varied between tumor DNA and nontumor DNA from the same patient. Peltomaki, et al [25], and Aaltonen et al [28] discovered that most CRCs from HNPCC patients showed microsatellite instability, and used the term "replication error positive (RER+)" to describe such tumors. The high frequency of microsatellite instability in HNPCC-related CRC suggested that the HNPCC gene might be a human homologue of the DNA mismatch repair genes previously described in yeast and bacteria [68].

Two excellent summaries of the DNA mismatch repair (MMR) have been published [69, 70]. Briefly, DNA replication fidelity is enhanced by a system that identifies, excises, and corrects mismatched sequences. Mismatches can arise during DNA replication either by incorrect base pairings (e.g., C-A rather than C-G) or by slippage of DNA polymerase on the template strand. Slippage is most likely to occur during replication of long repeating sequences. In *Escherichia coli*, where the process is best studied, three proteins are involved in mismatch repair: mutS, mutL, and mutH. The mutS protein binds to mismatched DNA sequences. MutH performs the crucial function of identifying the newly created DNA strand by virtue of differences in methylation between the tamplate and the new strand. (Without this function, there would be a 50% chance of repairing mismatches by replacing bases on the original strand rather than on the newly replicated one.) MutL and mutH then cooperate with mutS to remove the mismatched nucleotide(s). In humans, homologues to mutS (hMSH2 and GTBP) produce a heterodimeric complex that binds to mismatches, and mutL homologues (hMLH1 and hPMS2) form a heterodimeric complex that participates in MMR [71]. There is no mutH homologue in humans; repair of the correct strand appears to be directed by nicks in the newly replicated strand. Fishel et al [29], successfully cloned the first human homologue (hMSH2), and localized it to chromosome 2p21–22. At essentially the same time, a second group reported similar findings [53]. As described above, more than one gene contributes to DNA mismatch repair, and the syndrome of HNPCC can be associated with mutations in any of them.

In March 1994, two groups almost simultaneously reported the cloning of the mutL homologue (hMLH1) on chro-

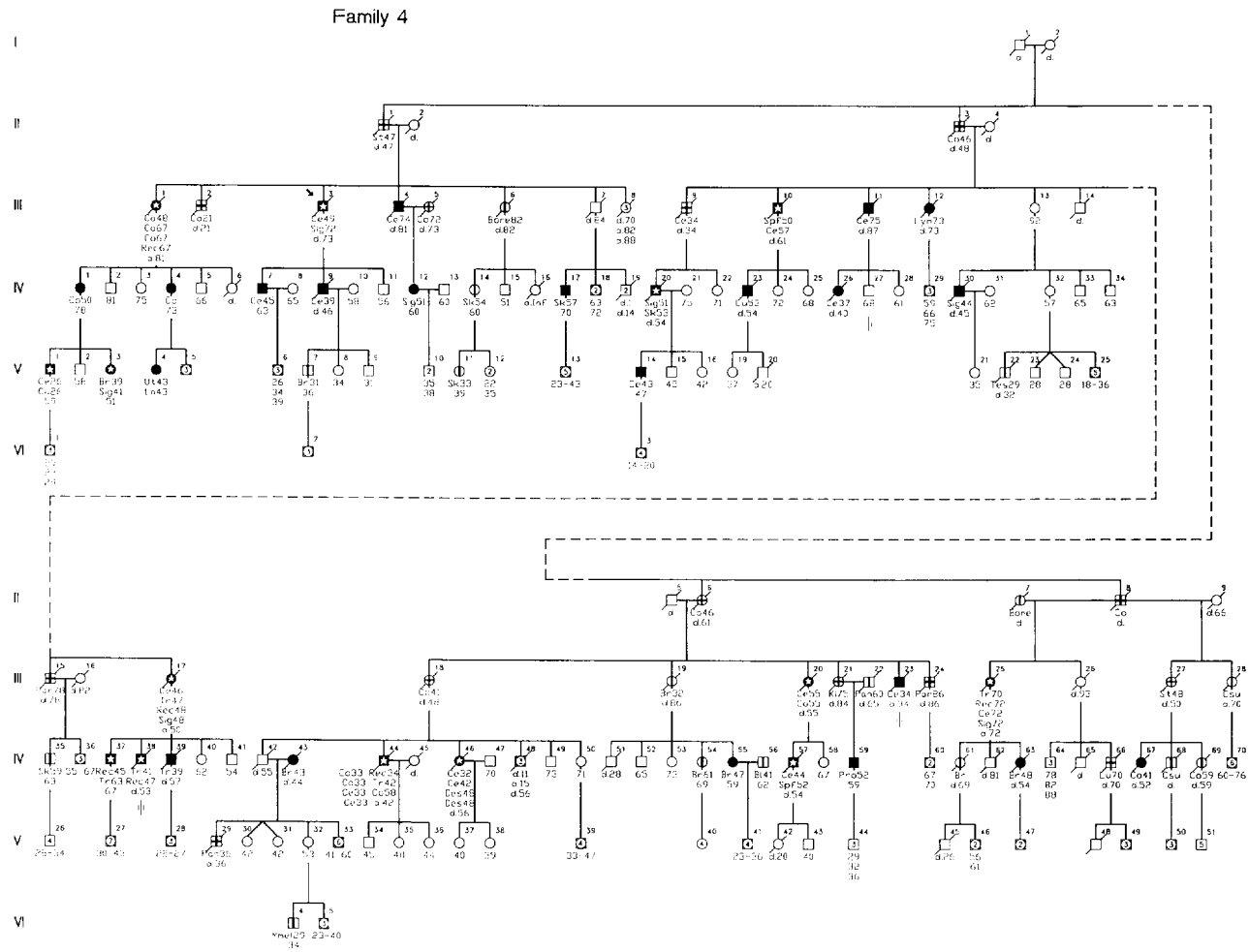


Figure 6 An updated pedigree of the first family that will later become designated as Lynch syndrome I. Note the paucity of extra colonic cancers. (Reproduced with permission. Lynch HT, Harris RE, Bardawil WA, Lynch PM, Guirgis HA, Swartz MJ, Lynch JF: (1977): Arch Surg 112:170–174).

mosome 3p21 [30, 31]. Other mutL homologues (hPMS1-2) and a mutS homologue (GTBP) [72, 73] have since been identified.

How is a germline mutation of a DNA mismatch repair gene related to the colorectal and extracolonic cancers of HNPCC? The working hypothesis is that mismatch repair genes function like tumor suppressor genes, so that heterozygous cells have normal or nearly normal repair activity, but loss or mutation of the wild-type allele in persons who inherit a mutation of the other allele results in cells with defective mismatch repair. For example, Hemminki et al [74] found nonrandom loss of heterozygosity (LOH) in tumors from HNPCC patients with a germline mutation in MLH1. In every affected case, the wild-type allele was involved, supporting the idea that a mutation in the wild-type allele is associated with the development of malignancy. In vitro work with colon cancer cell lines also supports the hypothesis: Parsons et al. [75] and Papadopoulos et al. [31] found that CRC line HCT116, which has mutations on both alleles of hMLH1, is deficient in mismatch repair. Umar et al. [76] showed that a different CRC line (LoVo) with two defective hMSH2 alleles also has repair-deficient cells. Koi et al. [77] were able to restore normal repair activity to a human colon cancer cell line known to have mismatch repair by inserting a normal copy of human chromosome 3.

In 1991, Loeb [78] suggested that the multistage model of carcinogenesis required more mutations than could reasonably be expected of cells subject to the observed spontaneous mutation rate. He predicted that an early mutation in a multistep process must be one that confers a "mutator" phenotype on affected cells. Repair-deficient cells qualify as a "mutator phenotype:" They accumulate mutations at a prodigious rate. Yeast with defective MMR may have 700 times as many mutations as repair-proficient yeast [68] and the frequency of mutations in human cells may be increased 1000-fold [79, 80].

What are the crucial mutations that must be acquired and how do they encourage malignant transformation? Early evidence suggests that, although cancers with microsatellite instability do accumulate mutations in the "usual" oncogenes and tumor suppressor genes (K-ras, p53, etc.) as spo-

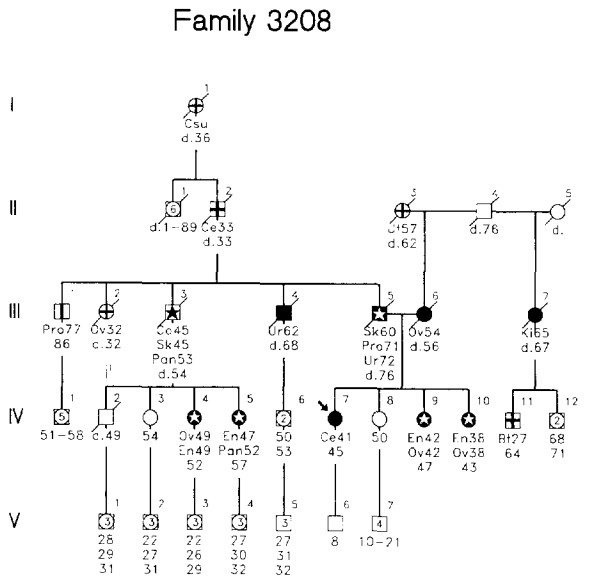


Figure 7 Updated pedigree of a family showing early onset synchronous carcinoma of the endometrium and ovary in two sisters and a paternal cousin. The proband had early onset colon cancer. Extending the paternal lineage led to a Lynch syndrome II diagnosis. (Reproduced with permission. Lynch HT, Cavalieri RJ, Lynch JF, Casey MJ (1992): Gyn Oncol 44:198–203).

radic CRC [81, 82], there may be mutations characteristic of HNPCC. One candidate was described by Parsons et al [83] who found that 90% of CRCs with microsatellite instability have mutations of the transforming growth factor Beta type II receptor (RII) gene. TGF-B inhibits the growth of colon epithelial cells; if the receptor is inactivated, growth inhibition cannot be accomplished. This particular receptor may be preferentially mutated in patients with defective mismatch repair because the gene contains a sequence of ten consecutive A bases; as noted above, long-repeat sequences are particularly prone to mismatch errors.

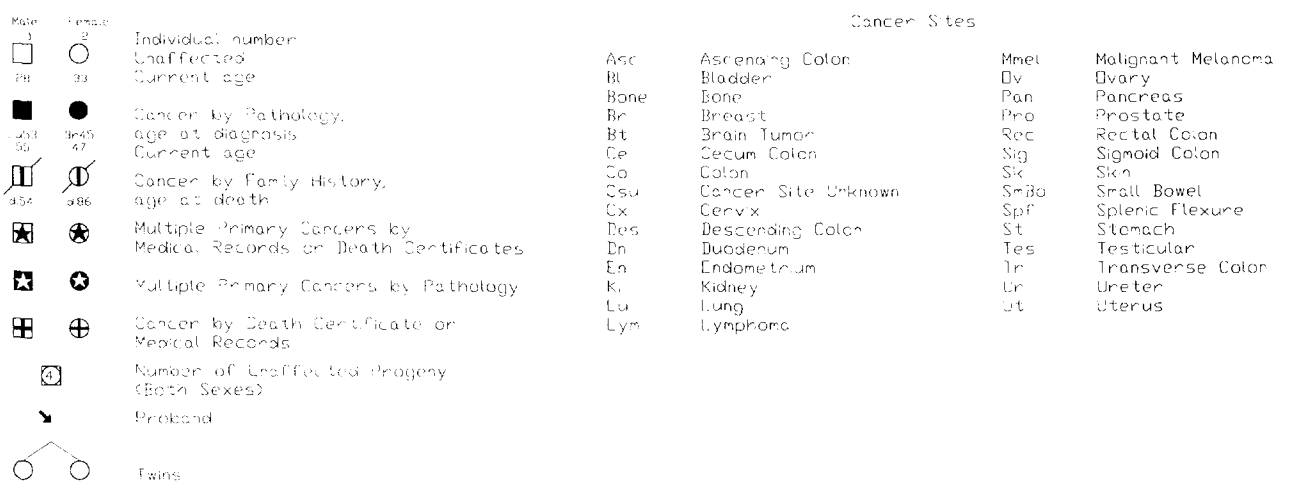
In addition to conferring "mutator phenotype," defective mismatch repair genes may affect the cell cycle. Hawn et al [84] studied a cell line (HCT116) with microsatellite instability that had no normal copies of the hMLH1 gene. Transferring a normal copy of human chromosome 3 into the cell line eliminated microsatellite instability, which was the expected result, but there was an additional change. The original cell line continued to grow after exposure to mutagens MNNG or 6-thioguanine, but the chromosome 3-corrected cell entered into cell cycle arrest and died. Flow cytometry demonstrated that the cells arrested at the G2/M check point, not at G1/S as is seen after radiation exposure or other injury. The authors proposed that DNA mismatch repair genes have a role in control of the cell cycle because they repair newly synthesized DNA, and they perform their checkpoint function at the G2/M boundary.

A recent animal study offers evidence that defective MMR is sufficient to produce malignancy. Reitmair et al [85] generated viable and fertile homozygous MSH2 deficient mice. Beginning at two months of age, most mice developed high-grade lymphoma; the tumors showed microsatellite instability. The authors suggest that mutant mice will be excellent models for the study of tumor progression and for screening carcinogens and anticancer drugs.

Risinger et al [86] have identified a 4-bp frameshift mutation in the hMLH1 gene that segregates with disease in affected members of a large HNPCC kindred. In one family member with breast cancer, the mutant allele was expressed in the malignancy, but both alleles (one wild-type and one mutant) were observed in her normal breast tissue. The breast cancer exhibited widespread microsatellite instability, as did breast cancers obtained from several other HNPCC kindreds.

These observations suggest that even though statistical analysis of a large series of HNPCC families did not show an excess of breast cancer [65], phenotypic heterogeneity may occur, producing a subset of HNPCC kindreds with susceptibility to breast cancer. Such subsets could result from genetic or environmental factors. For example, specific mutations in the large MLH1 or MSH2 gene might in-

Figure 8 Legend to figures 3–7.



flict a predisposition to breast cancer. In support of this idea, the 4-bp mutation described above is similar to a 4-bp mutation located 67 bp downstream in the hMLH1 cDNA sequence described previously [31]. The latter pedigree was notable for three cases of breast cancer, two of which occurred in individuals at age 28 and 36 years [87].

Risinger et al [86] conclude that NMR gene mutations may be etiologic in hereditary breast cancer and that breast cancer represents an integral tumor in the Lynch syndrome II variant of HNPCC. However, the penetrance of this genetic defect contributing to breast cancer must be lower than that for carcinoma of the colon and extracolonic cancers in Lynch syndrome II families because breast cancer does not occur with a statistically significant higher frequency in HNPCC families than in the general population [65].

Surveillance and Prophylactic Colectomy in HNPCC

The natural history of the Lynch syndrome indicates that CRC occurs at an average age of 44. Seventy percent of these lesions are located in the proximal colon. A subtotal colectomy should be performed as opposed to a hemicolectomy or a segmental resection because there is a 45% risk for a second or third primary cancer of the colon over ten years (Table 1). Because of the proximal distribution of CRC, we recommend that gene carriers begin a screening colonoscopy regimen by age 20–25 (the youngest patient with CRC in HNPCC is age 13); and we repeat this procedure every one to two years to age 35, and annually thereafter.

Gene Linkage and Identification of HNPCC Candidates for Colonoscopy

Piepoli et al [88] evaluated the importance of gene linkage findings to identify HNPCC patients who are at increased cancer risk. The study involved linkage analysis in an extended HNPCC kindred. There were 61 blood relatives in this extended family, five of whom had CRC. Twelve unrelated family members also underwent DNA sampling for genetic analysis. All five affected individuals had a haplotype with the same alleles seen in 13 first-degree healthy gene carriers. Six asymptomatic gene carriers were screened, yielding one incidental CRC and four adenomas. An adenoma was found in 1 of 17 noncarriers. The authors note that "the remaining 16 noncarriers have undergone 67 unnecessary colonoscopies." Linkage analysis was capable of differentiating gene carriers from nongene carriers in this family so that CRC screening could be limited to the gene carriers, leading to a saving of time and cost.

Unfortunately, not all patients comply with surveillance recommendations. Poor compliance with colonoscopic surveillance may be an important reason for recommending the option of prophylactic colectomy in HNPCC germline carriers. Reasons for poor compliance may include lack of funds, fear of insurance discrimination, or fear and anxiety about finding a lesion. An attempt should be made to ameliorate these concerns prior to recommending surgical prophylaxis.

Prophylactic Subtotal Colectomy and HNPCC Germline Carriers

It is important that surgeons consider the option prophylactic subtotal colectomy for their patients who are germline carriers for HNPCC. Clearly, all germline carriers will require genetic counseling. They need to be educated about all of the facets of HNPCC's natural history, with particular attention to the limitations of colonoscopy, the potentially rapid rate of cancer progression, the penetrance of the germline mutation, and the potential sequelae of this surgical procedure. Patients who have undergone subtotal colectomy must be told that they will require lifelong endoscopic evaluation of their rectal segment.

DeCoss [89], in discussing prophylactic surgery in the Lynch syndromes, asks whether the level of risk in the disorder merits preventive colectomy in a patient at approximately 50% risk for cancer based on the autosomal dominant genetic model, but who has not yet developed cancer. His answer to this question is in full accord with our position as he states "In the added presence of defective HNPCC genes, the answer seems affirmative." With respect to a woman with the Lynch syndrome II variant in whom surgery is planned for the presence of CRC, he suggests prophylactic bilateral oophorectomy and hysterectomy, particularly if the woman is postmenopausal.

Finally, it must be realized that the issue of prophylactic surgery in HNPCC is not significantly different from the well accepted prophylactic surgical approach for FAP. In FAP, the average age of CRC onset is about 39 years, whereas in HNPCC it is about 44 years. CRC occurs with approximately the same frequency in germline carriers of the two syndromes. The only difference is that in FAP, we have the florid polyposis phenotype to guide us.

Ideally, a case-control study is needed to assess the acceptability and cancer control potential of prophylactic subtotal colectomy versus colonoscopy in germline carriers. However, given certain ethical concerns, particularly our experience with interval CRCs, and the hypothesis of accelerated colorectal carcinogenesis in HNPCC, it might be difficult to develop such a study and to assure compliance when patients are made fully aware of all of the facets of the natural history of the Lynch syndrome.

Aggressive Adenomas in HNPCC

There is evidence that adenomas in HNPCC progress to carcinoma at a more rapid rate than occurs in the general population. Muto et al [90] suggest that the rate of polyp progression to carcinoma in the general population is usually quite slow, with an estimated course of 10 to 15 years. It is postulated that loss of DNA repair proficiency in HNPCC may have little or no effect on tumor initiation, but that progression is accelerated [91–94]. A Finnish study has provided the best evidence for the concept of "aggressive adenomas" in HNPCC [95]. The authors found and removed 22 adenomas from the group under surveillance; compared to a group that refused surveillance, an estimated 7.8 cancers were prevented, or one for every 2.8 polypectomies. In contrast, the National Polyp Study indicates that 41 to 119 polypectomies are necessary to prevent one cancer [96, 97].

Interval Cancers and Colonoscopy

We have a number of anecdotal reports of interval cancers occurring in HNPCC patients within 1 to 3.5 years following surveillance colonoscopy. One recent example (not

published) involves a woman from an HNPCC family who had endometrial carcinoma at age 36. She underwent colonoscopy every two years. Eighteen months after a normal colonoscopic evaluation, she was diagnosed with a Stage B1 (Astler-Coller) cancer of the transverse colon. She underwent a segmental colonic resection. At that point, she insisted on colonoscopy every six months. Five months following colonoscopy, she was found to have two primary colon cancers, one Stage B1 in the cecum, and the second a Stage A in the low rectum.

Lanspa et al [98] studied 225 individuals with 313 colon cancers from families on file in the Creighton Lynch syndrome resource. Six patients from different families had colon cancers arising within 4 1/2 years of colonoscopic surveillance. Another seventeen patients had metachronous colon cancers within five years of resection (less than subtotal colectomy) of their first colon cancer. Thus, of 225 CRC patients from Lynch syndrome families, 10.2% had CRC within five years of colonoscopy or colon resection.

GENETIC COUNSELING AND HNPCC

The Physician and Genetic Counseling

When families are recognized as HNPCC and predictive germline testing is being considered for MSH2, MLH1, PMS1, and PMS2 status, one must consider the availability of genetic counseling. Counseling should be initiated prior to individual DNA testing. Patients need to be told the pros and cons of undergoing genetic testing and they need considerable emotional support and understanding when they receive the test results; it is essential that follow-up counseling is available. Unfortunately, there are a limited number of certified genetic counselors who are knowledgeable about cancer genetics. If physicians are to fill this void, they must recognize that there is more to genetic counseling than merely imparting genetic risk information.

The physician faces a major responsibility in this challenging area of DNA testing and genetic counseling. Some general issues are as follows: 1. Although accomplishments through molecular genetic research have been momentous, the physician will remain the most important figure in the diagnosis and management of HNPCC. 2. However, because of the supply and demand principle, cottage industries are likely to develop and flourish. Physicians will need to be "gatekeepers" and understand the significance of cancer genes, their case finding potential, and how to respond clinically to the natural history features of hereditary cancer so that appropriate cancer control options can be exercised. 3. Finally, it must always be kept in mind that the patient is the one to decide whether testing is acceptable and whether or not the surveillance and management considerations are to be pursued.

PSYCHOLOGICAL ASPECTS OF HNPCC

Quality of Life and Health Behavior

As our knowledge of HNPCC risk factors has increased, so has our attention to the impact of risk assignment on the patient's quality of life.

For those persons who ultimately do decide to receive genetic testing for HNPCC susceptibility, there may be a significant burden associated with the knowledge that one is a carrier of a cancer-predisposing mutation. Progress in molecular genetics, however, has significantly out-paced the physician's ability to translate this new information to the patient's advantage. Society in general has also lacked preparedness, as evidenced by many unresolved ethical problems and certain discriminatory practices by insurers and employers. Personal problems of intra family strife, fear, and anxiety may occur relevant to the outcome when told the good news, namely germline mutation negative status, but with the potential for survivor guilt, versus the bad news, being positive for the cancer prone mutation and the almost inevitable cancer outcome. Research directed at each of these concerns is needed. Undoubtedly, a potpourri of other problems are certain to arise.

ASCO Position on Genetic Testing for Cancer Susceptibility

The American Society of Clinical Oncology (ASCO) [99] has recently published a position paper dealing with genetic testing for cancer susceptibility. This statement recognizes the role of clinical oncologists in documenting a family history of cancer and in providing counseling regarding the patient's inordinately high cancer risk and the need to provide options for prevention and early detection in those families where genetic testing may aid in the genetic counseling process. Whenever possible, this genetic testing should be performed in the setting of long-term outcome studies.

ASCO also recognizes the need to provide educational opportunities for physicians relevant to genetic testing and genetic counseling. Informed consent by the patient is an integral part of the process of genetic predisposition testing, whether on a clinical or research basis. Predisposition testing should be performed only when there is a strong family history of cancer indicative of hereditary etiology, where the results can be adequately interpreted and where there is a potential to aid in the patient's medical management. Importantly, ASCO urges oncologists to utilize those laboratories that are committed to the validation of the testing methodologies in context with an approach that will facilitate families' participation in long-term outcome studies.

Patients must be cognizant of the advantages, as well as limitations of early cancer detection and prevention modalities. ASCO also recognizes the need to strengthen regulatory authority over laboratories that provide cancer predisposition tests that will ultimately be utilized for informed clinical decisions. In the interest of protecting patients/families, ASCO endorses the adoption of legislation to prohibit discrimination by insurance companies or employers based on the individual's susceptibility to cancer. Finally, ASCO endorses the need for all individuals at hereditary risk for cancer to have appropriate genetic counseling covered by public and private third-party payers.

Should DNA Testing Be Put on Hold?

Some groups have suggested that DNA testing for genetic disorders be temporarily halted. Several documents issued

in the United Kingdom [100], the United States [101], and the Netherlands [102] have emphasized the need for caution when translating molecular genetic knowledge into the clinical practice setting. These admonitions are related to cancer genetic susceptibility testing [103], as well as the genetic testing of children [104]. In reviewing this subject, Harper [105] noted that several European countries, as well as the European Union, have considered introducing laws to regulate genetic tests, whereas in Norway some laws have already been enacted [106].

Caution in implementing any new discipline is worthwhile. However, we believe there is a responsibility to provide DNA information to informed and consenting HNPCC family members. How can we deny them the knowledge that could save their lives?

Results of DNA-Based Genetic Counseling in HNPCC

To date, we have counseled three HNPCC families who carry either MLH1 or MSH2 mutations and have offered them the opportunity to receive their individual test results. This population was comprised of 179 individuals, 55 of whom were gene positive, 97 gene negative, with results pending on 27 patients. Of this group, 79 have been counseled and given their gene status. The discrepancy between the number of individuals who have firm DNA findings (gene positive or gene negative) and those who have come for counseling is due in part to factors that include fear, anxiety, and apprehension about finding out about their results, as well as concern about insurance discrimination.

Results have shown that there were two primary reasons patients sought their risk assessment: 1) to inform their children and other family members of potential cancer risk; and 2) to make informed decisions regarding their own future surveillance. Interestingly, 42% of the gene positive family members counseled viewed prophylactic colectomy as a viable method of cancer prevention, whereas the remainder favored aggressive surveillance (annual colonoscopy). Only 20% of the gene positive women considered prophylactic hysterectomy a reasonable method of prevention. One-fourth of the gene positive individuals expressed concerns about insurance discrimination. These preliminary findings are in preparation for publication.

SUMMARY AND CONCLUSIONS

Prodigious advances in molecular biology and genetics are significantly changing how hereditary CRC and its integral extracolonic cancers are diagnosed and managed. We have reviewed the salient aspects of FAP and HNPCC but, given the rapid pace of the information explosion in our midst, some of this information will have become obsolete the minute we have rested our pen.

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We dedicate this paper to our late colleague, Lemuel Herrera, M.D.

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