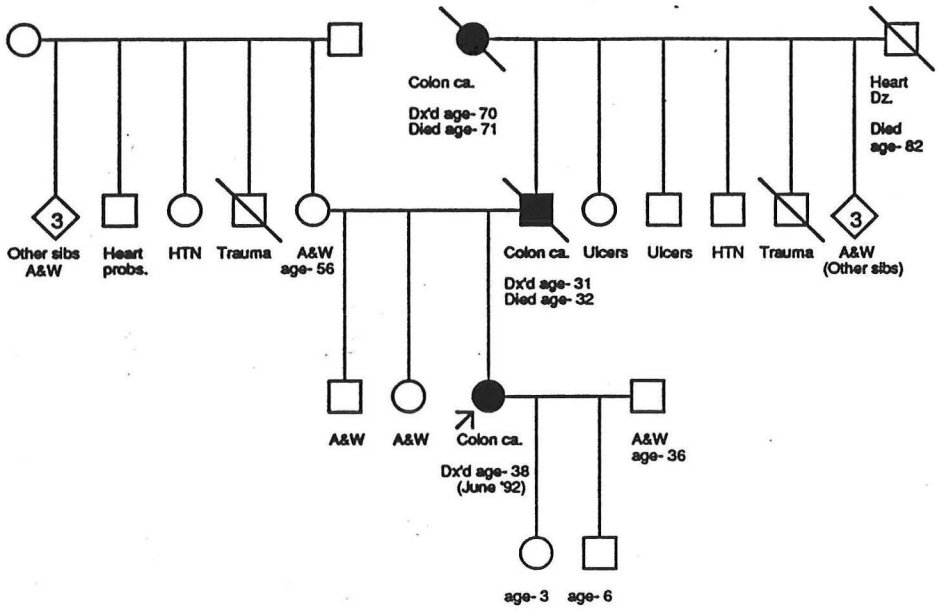
Hereditary Predisposition to Colorectal Cancer: New Insights



Medical Grand Rounds
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1. INTRODUCTION

Colorectal cancer (CRC) is a serious public health problem in this country. We will diagnose approximately 7200 new cases in Texas in 1993 (1). About 60% of these patients will present with regional or metastatic involvement. Although 5 year survival is 90% for localized disease, it falls to 58% and 5% for regional and metastatic cancer. These statistics illustrate the clear rationale for early detection and treatment advocated by major national agencies (2,3). Perhaps the most effective approach to prevention would be presymptomatic detection of individuals genetically and/or culturally destined to develop CRC. In this Grand Rounds, I will review recent progress toward this still distant goal, concentrating primarily on hereditary nonpolyposis colorectal cancer (HNPCC). Recent identification of a new HNPCC gene will soon change our approach to screening for common (sporadic) CRC as well as for HNPCC.

2. ETIOLOGY OF CRC

2.1 Definable genetic or familial predisposition

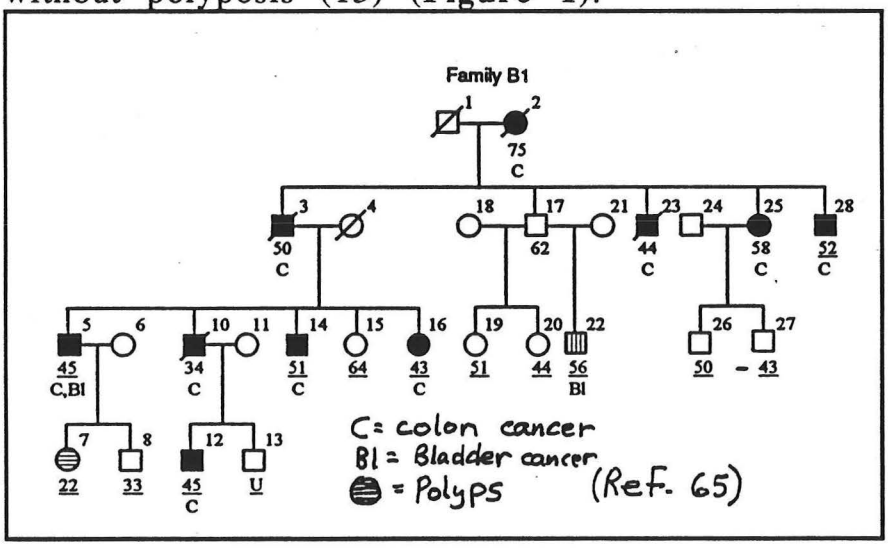
As with most diseases, heritable and environmental factors interact in the pathogenesis of CRC. Increased genetic and/or familial risk can be identified in 3 clinical circumstances (**Table 1**). These are the polyposis syndromes, HNPCC and common (sporadic) CRC.

Table 1. Familial Risk in Colorectal Cancer

Syndrome	Variants	Risk to First-Degree Relatives	Predisposing Gene
Familial adenomatous polyposis(FAP)	Famlial polyposis	50%	*APC* (5q)
	Gardner's syndrome, Turcot's syndrome, Oldfield's syndrome, Heriditary flat adenoma syndrome		
Heredity nonpolyposis colon cancer(HNPCC)	Lynch I	Up to 50%	*FCC* (2p)
	Lynch II		Other Undefined Genes
	Unnamed variants		
Common (sporadic) CRC	{Heterogeneous}	Heterogeneous, average 2-fold increased risk	Unknown; probably many genes

2.1.1 *Polyposis syndromes.* Dr. Gordon Luk reviewed the first of these, familial adenomatous polyposis (FAP), in his Grand Rounds in September of last year. FAP is an autosomal dominant disease of several variant forms, all caused by inactivating mutations of the *APC* gene on chromosome 5q (4-6). In the classical form, hundreds to thousands of adenomatous polyps appear in the colorectum beginning early in adolescence, and almost all affected individuals develop CRC by age 40. FAP accounts for <1% of all CRC. Gardner's syndrome, Turcot's syndrome, Oldfield's syndrome and various minimal polyposis syndromes such as the hereditary flat adenoma syndrome (7-9) are probably all variants of FAP and have been linked to chromosome 5q (10-12). Importantly, the flat adenoma syndrome and perhaps other types of minimal ("forme fruste") polyposis may present with fewer than 100 polyps and thus be confused with HNPCC or sporadic neoplasia.

2.1.2 *Hereditary nonpolyposis colorectal cancer (HNPCC).* HNPCC describes a heterogeneous collection of familial predispositions to CRC, defined by the presence of at least 3 first degree relatives in 2 generations with CRC and without polyposis (13) **(Figure 1)**.



HNPCC is responsible for about 5% of all CRC. In some kindreds, only colon cancer is found and most of the cancers originate proximal to the splenic flexure (Lynch I syndrome). Other families are afflicted with randomly scattered colorectal tumors (14). In still other families, the disease may manifest as colon cancer and/or as other kinds of adenocarcinomas including endometrial, ovarian, gastric, duodenal or proximal ureter/renal pelvis carcinomas (Lynch II syndrome) (15,16). HNPCC patients form adenomatous polyps at about the same frequency as people with sporadic CRC (17).

Familial clustering of cancer in HNPCC suggests a genetic etiology, and therefore one would like to exploit these families to track down the

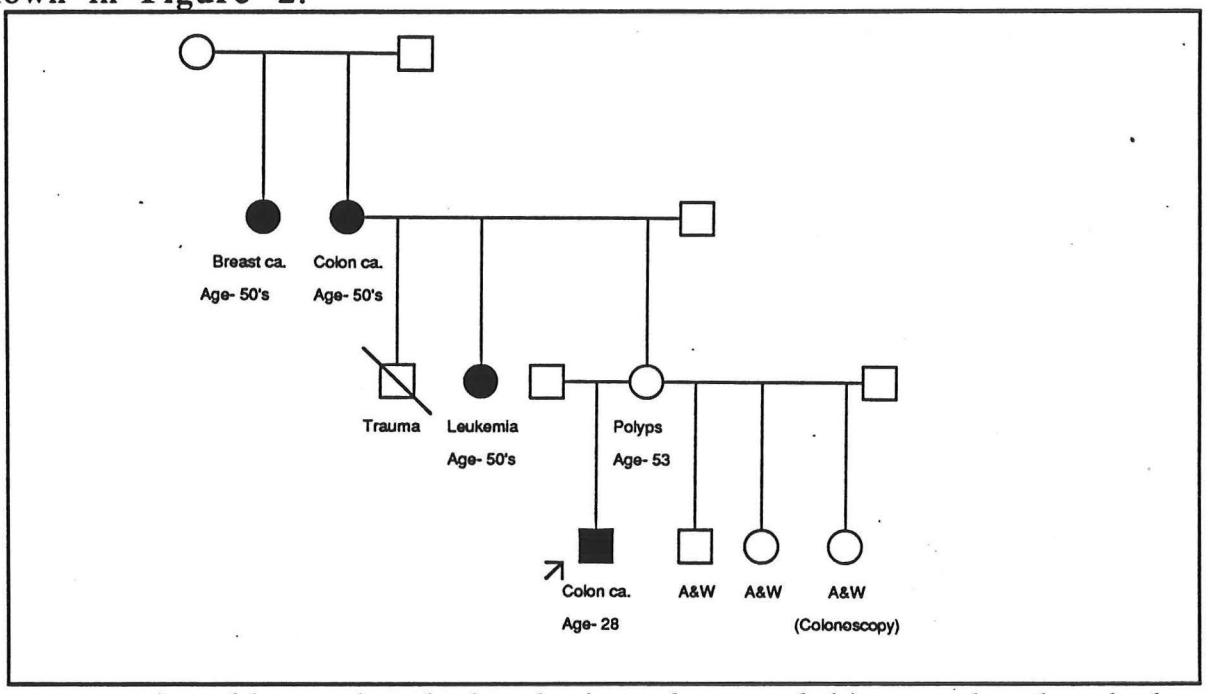
responsible genes. However, problems with ascertainment may arise which reflect the high incidence of sporadic colorectal cancer and the heterogeneity of HNPCC. Thus, clustering of cancers in some families may result from shared environmental influences or chance aggregation of cases rather than from genetic predisposition. If these nongenetic factors are responsible for family clustering, genetic linkage analysis will be fruitless. Even if heritable factors are the explanation, low-quality pedigree information because of small families, mistaken paternity, incomplete geneologic data and eary death of relatives limit the power of linkage analysis. Other problems frustrating this analysis include incomplete penetrance in younger relatives, phenotypic confusion with a polyposis syndrome and linkage heterogeneity. Therefore, for genetic studies as well as clinical management of families a detailed, documented family history is essential, including at least all first degree and selected second degree relatives such as both sets of grandparents, aunts and uncles. Older relatives are the most phenotypically informative. Documentary evidence of nature of any tumors, their location and age at diagnosis and the presence of synchronous or metachronous malignancies should be obtained. Detailed information on colonic polyps, including number, location and age at first detection is also essential. Physical examination must include colonoscopy, noting the number, location, size, appearance, and histology of any polyps (13).

When the family history falls short of HNPCC criteria, three clinical clues regarding the index case should raise the suspicion of increased family risk: age at diagnosis < 40 yr, multiple synchronous or metachronous primaries and the occurrence of CRC and a second primary carcinoma of the Lynch syndrome II type (most commonly endometrial cancer).

The following case histories, recently encountered at Parkland Hospital, illustrate the variety and some of the problems in characterization of familial CRC risk .

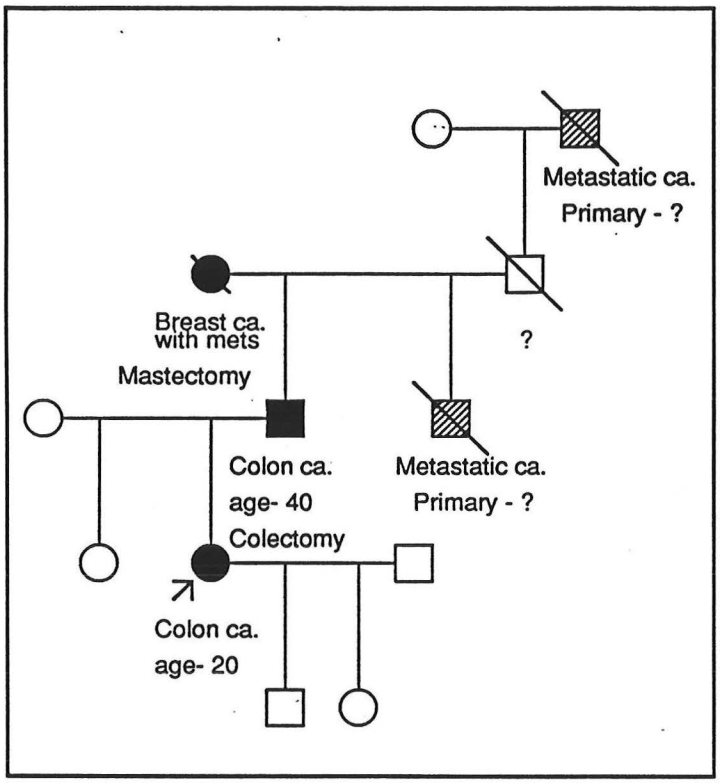
RR is a 29 year old white male who underwent resection of a Duke's C2 sigmoid cancer, subtotal colectomy and ileosigmoid anastomosis in March, 1992 (age 28) at Harbor Hospital, Torrance, California. Histopathology was poorly differentiated adenocarcinoma. The remainder of the colon was normal; no adenomas were identified. Eighteen of 18 examined lymph nodes, including para-aortic nodes, contained tumor. In February 1993 he felt abdominal and rectal pain, his stools increased in frequency and by May he lost 15 lbs. Colonoscopy revealed a near-obstructing rectal mass which was opened with the tumor probe. Histology was again poorly differentiated adenocarcinoma. He is now receiving external beam pelvic

irradiation. His pedigree is shown in **Figure 2.**



To summarize, his mother had colonic polyps and his grandmother had colon cancer in her early 50's. His mother's sister had leukemia at age 50 and his grandmother's sister had breast cancer in her 50's. We are seeking additional medical records and are still uncertain whether this family represents HNPCC or a minimal polyposis syndrome.

TB is a 20 year old black woman who presented with right lower quadrant pain. A sonogram suggested a periappendicial abscess. After percutaneous drainage and subsequent perforation, emergency exploration revealed a large mass associated with her cecum and proximal ascending colon which were resected. Although the extent of exploration and resection were limited, no polyps were seen. Pathology revealed a fungating mass 7.5 cm in diameter with central necrosis, invading through muscularis propria into the pericolic fat. Microscopically, areas of well- and poorly differentiated mucin-containing adenocarcinoma were present. One of 13 regional nodes contained tumor. The pedigree is shown in **Figure 3.**



Her father had a colectomy at age 40. His brother and grandfather died of metastatic carcinoma. The father's mother had a mastectomy and died of metastatic cancer. Although data are incomplete, her young age, the right-sided tumor and the absence of polyps in the resected specimen suggest HNPCC.

2.1.3 *Sporadic colorectal neoplasia.* Several studies have shown that the risk of colorectal neoplasia in first-degree relatives of patients with common (sporadic) CRC is increased about 2-fold (18-23). Risk to first-degree relatives increases with decreasing age of CRC patients and increases to about 6-fold for people with two affected relatives (23). Risk to spouses of CRC patients and spouses of controls is about equal, suggesting that shared environmental influences are less important than genetic factors as determinants of observed familiality (22,23). The cumulative incidence of CRC as a function of age increases more steeply in relatives of CRC patients than in relatives of controls, and the slopes of these curves increase with decreasing age of onset of CRC in the index patients (23) **(Table 2)**.

Table 2. Cumulative Incidence of CRC in First-Degree Relatives of Patients with Common (Sporadic) CRC

Patient Category	Age (Yr) of First-Degree Relatives				
	40	50	60	70	80
	(Cumulative Risk in Percent)				
1. All CRC case patients	0.2	0.7	1.8	3.3	5.9
2. Case patients diagnosed ≤55yr	0.2	0.9	2.8	5.0	8.4
3. Case patients diagnosed ≥55yr	0.1	0.5	1.0	2.2	4.4
4. Control patients (without CRC)	0.0	0.1	0.6	1.3	2.4

From Reference 23.

These data support the contention that screening of first degree relatives of CRC patients should begin at age 40, about 10 years earlier than for people without a family history of CRC. In summary, predisposition to common (sporadic) CRC is likely to be influenced by multiple undefined genes, and the magnitude of risk depends on the age and number of relatives with CRC.

2.1.4 *Can we capitalize on genetic determination of risk to focus screening efforts?* If we could presymptomatically identify people at high risk of CRC, intensive screening could be restricted to carriers, in whom appropriately timed pre-emptive resection would prevent cancer mortality (secondary prevention). In FAP families segregating known mutant *APC* alleles, this is now possible. I will show examples of FAP carrier detection later on. In HNPCC and common (sporadic) CRC, until we know the genes involved, prospective identification of family members at greatest risk is not feasible.

2.2 Environmental factors in colorectal carcinogenesis

Based on epidemiologic evidence, experimental animal studies and analyses of enteric contents, environmental or lifestyle factors, probably mainly dietary, have long been thought to play an important role in CRC development. The epidemiologic evidence falls into several categories:

geographic studies, population migration studies and other comparative population analyses (**Table 3**).

Table 3. Evidence Supporting Environmental (Dietary) Factors in Colorectal Carcinogesis

Type of Study	Group at Risk	Highest/Lowest Risk	Reference
Geographic comparisions	Western populations	2-3	24, 25
Geographic comparisions	High fat, low fiber consumers	3-5	25, 26
Migration Studies	First-generation oriental migrants to U.S.A.	2-3	27,28
Cohort	High Fat, red meat	2.5	31

People in western countries have 2-3 times the risk of CRC as do Chinese, Japanese and most residents of developing countries (24,25). Across many countries, CRC risk and diets high in fat and low in fiber are strongly correlated (25,26). Within their first generation of residence, Japanese who migrate to Hawaii experience a 2-3 fold increase in CRC risk, which approximates the risk in white Hawaiians (27,28). In this country, standardized mortality ratios for CRC among Seventh-Day Adventists and Mormons, each of whom practice dietary modification, are consistently lower than those of control groups and the population at large (29,30). In the Nurses Health Study, diets high in animal fat and colon cancer incidence were strongly correlated, even after adjusting for total calorie intake (31). Mutagens such as fecapentaenes are present in human stools, and their concentrations have been correlated with CRC risk in South African populations (32,33). Even in FAP, where genetic determination is paramount, colonic polyps may regress after diversion of the fecal stream (34). This suggests that enteric contents contain promoters of neoplasia.

In the context of today's discussion, these findings have two important implications. First, lifestyle and dietary factors may partly explain the variable penetrance of CRC in genetically predisposed groups. Second, if adopting a Western-style diet can result in a 3-fold increased risk of CRC within a single generation, then we can reasonably expect that appropriate dietary modifications or chemoprevention regimens capable of reducing

risk by a like amount can be found. This is the primary prevention strategy. Since these measures impose some burdens, costs and possibly even risks, ideally we would like to recommend them only to people at significant risk.

3. BIOMOLECULAR PATHOGENESIS OF CRC

3.1 Common (sporadic) CRC and FAP

Many clinical and experimental observations have defined a common stepwise series of events in CRC pathogenesis (34). The first observable event is an expanded zone of epithelial cell proliferation in colonic crypts (35,36). Clonal proliferation eventually forms a polyp. Further progression leads to carcinoma in situ. As the polyp enlarges, the probability of invasion through basement membrane increases. Growth may be primarily fungating with ulcer formation, or penetrating and circumferential. Next, lymphatic and hematogenous metastases disseminate the tumor. In FAP, inherited loss of function of one *APC* allele greatly increases the probability of formation of adenomas, each of which becomes an engine of neoplastic progression inevitably leading to one or more carcinomas.

At the genomic level, a surprising amount of molecular damage accompanies this process (37-39) **(Table 4)**.

Table 4. Growth-Promoting and Growth-Restraining Genes Commonly Mutated in CRC

Gene	Function	Effect of Mutation	Fraction of Carcinomas with Mutations	Timing of Mutations in Tumor Progression
I. Growth-Promoting Genes				
RAS	Signal transduction	Activating	30-50	Early to Mid
MYC	Transcriptional regulation	Activating	2	?
Cyclins D,E	Cell cycle progression	?	4	?
II. Growth-Restraining Genes				
APC	Cell membrane location	Inactivating	>70	Early Mid or Late
P53	Transcriptional regulation	Inactivating	>70	Late
DCC	Cell adhesion molecule	Inactivating	>70	Mid to Late

Early: adenoma; Mid: adenoma with carcinoma in situ; Late: invasive cancer

Some of the genetic lesions activate growth-promoting genes such as *K-RAS* (40), *MYC* (41) and cyclin (42) alleles by point mutation, hypomethylation or amplification. Mutated *K-RAS* alleles can easily be detected in stool samples from patients with adenomas and CRC, suggesting a new approach to screening (43). K-RAS protein transmits growth signals to intracellular targets when it binds GTP, and the signal is terminated by GTP hydrolysis (44,45). Mutated forms of RAS hydrolyze GTP inefficiently and thus the growth signal is locked "on". Such mutated forms are found in 30-50% of colorectal adenomas and carcinomas (40). Right now mutated K-RAS is generating a great deal of excitement as a potential target for selective therapy of CRC and other human cancers. K-RAS must be bound to cell membranes in order to function. Inhibitors of membrane binding are now undergoing preclinical evaluation in the Goldstein/Brown laboratory on our Campus, in collaboration with Genentech.

Several known or candidate growth-restraining genes are inactivated during CRC progression, often by allele loss but also by other mechanisms. These include *APC* (chromosome 5q) (4-6), *P53* (chromosome 17p) (46) and *DCC* (chromosome 18q) (47). In FAP, germline mutations usually truncate the *APC* gene and encode nonfunctional protein (6,48). Although this "single hit" is sufficient for polyp formation (48), total loss of APC expression in polyps and FAP carcinomas has been reported in Japanese patients (49). Loss of APC function by somatic mutation or allele loss is also very common in sporadic CRC (6,40,48). APC is normally expressed on the basolateral colonic epithelial cell surface in increasing amounts as cells move up the sides of the crypts (48). Thus it may play a role in restraining proliferation of the superficial crypt and surface epithelial cells. In his Grand Rounds on April 22 of this year, John Minna reviewed possible functions of the P53 protein. P53 is a transcriptional regulator which may prevent genomically damaged cells from progressing forward through the cell division cycle, thus providing maximal opportunity for DNA repair before the lesions are fixed by DNA replication and mitosis (50-53). *P53* is lost by deletion late in the process of progression in most CRCs (46). The DCC protein is similar to known cell adhesion molecules, is expressed on the surfaces of normal colonic epithelial cells and may transmit growth-restraining signals between cells (47,54). Like P53, it is very commonly lost late in CRC progression (40,47,54).

These candidate tumor suppressor genes may only represent the tip of a large iceberg of gene loss in common (sporadic) CRC. Using chromosome-specific probes, Vogelstein has estimated that most chromosome arms

suffer loss of some alleles at frequencies ranging from 5 to 80% of the tumors examined (55) (**Figure 4**).

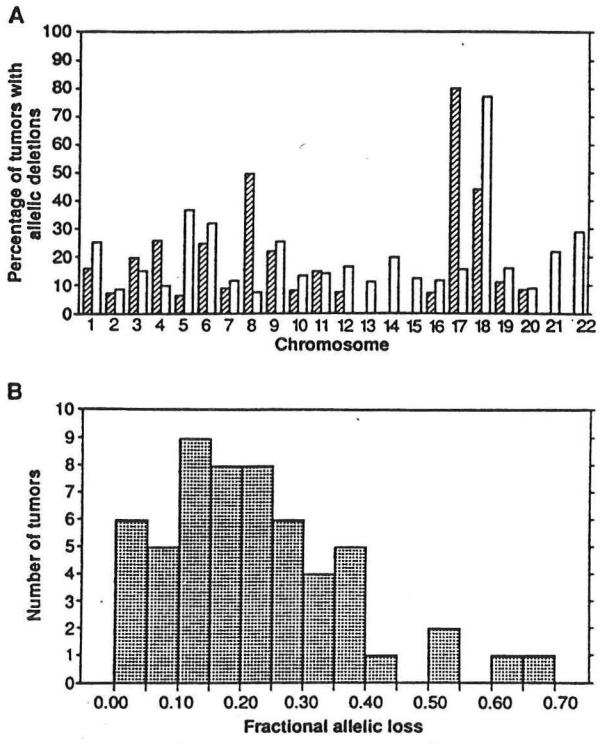


Fig. 4 (A) Frequency of allelic deletions in individual chromosomal arms. Allelic deletions were evaluated with RFLP analyses, examples of which are in Fig. 2. DNA from paired normal colonic mucosa and tumor tissues was cleaved with one of three enzymes (Taq I, Msp I, or Hind III), and evaluated with probes from each nonacrocentric autosomal arm. The probes used are listed (*23*), together with references describing their derivation and polymorphism patterns. Only informative tumors, that is, those in which DNA from the normal tissue exhibited a heterozygous pattern for one or more allelic markers from the indicated chromosomal arm, were used to determine allelic loss frequencies. The number of tumors informative for each chromosomal arm is listed in (*23*). An allelic loss was scored if an RFLP fragment present in normal DNA was lost in at least 80% of the neoplastic cells, as assessed by comparison of the autoradiographs with histologic evaluation of the cryostat sections from which the tumor DNA was purified. Open bars, q arm; hatched bars, p arm. (**B**) Frequency of allelic deletions in individual tumors. The FAL in each tumor was defined as the number of chromosomal arms on which allelic loss was observed divided by the number of chromosomal arms for which allelic markers were informative.

(Ref. 55)

Allele loss, usually detected as loss of heterozygosity, is central to the pathogenesis of FAP and common (sporadic) CRC (**Table 5**).

Table 5. Allele Loss in Colorectal Neoplasia

	Adenoma			Carcinoma	
Locus	Class I	Class II	Class III	FAP*	Sporadic
	(% of tumors with deletion)				
5q	0	29	29	24	36
18q	13	11	47	40	73
17p	6	6	24	31	75

From (40) and (40a). Class I: FAP-derived, small low-grade dysplasia. Class II: Non-FAP patients, moderate dysplasia, no carcinoma. Class III: contained carcinoma which was dissected off prior to analysis. *FAP carcinomas were not microdissected; thus figures could be underestimates (40a).

3.2 HNPCC

Two features suggest that the pathogeneses of HNPCC and of common (sporadic) CRC are not the same. First, the frequency of right-sided lesions is far more common in HNPCC than in common (sporadic) CRC (13,16). Second, HNPCC is more likely to be diploid or near-diploid than are sporadic CRC's or even adenomas (56,57). I will return to this topic later.

4. THE POWER OF LOCUS POLYMORPHISMS IN RISK ASSESSMENT

When no treatment for a genetic disease is available, as in Huntington's chorea, prescience of an approaching disabling and ultimately fatal disease is a mixed blessing at best. In the case of CRC, however, effective secondary prevention is available and primary prevention may be feasible. Carriers are identified by *APC* allele typing. For reliable results, allele markers must be within or extremely close to the *APC* gene so that genetic recombination between APC and the marker does not happen. Markers should be highly polymorphic so that most people are heterozygous. Several highly polymorphic markers very close to the *APC* gene have been discovered, the best being the C-A repeat (microsatellite) marker D5S346 (58). If a few family members with and without the disease are available, a conclusive determination of carrier status by allele identification can usually be made in presymptomatic children (**Figure 5**).

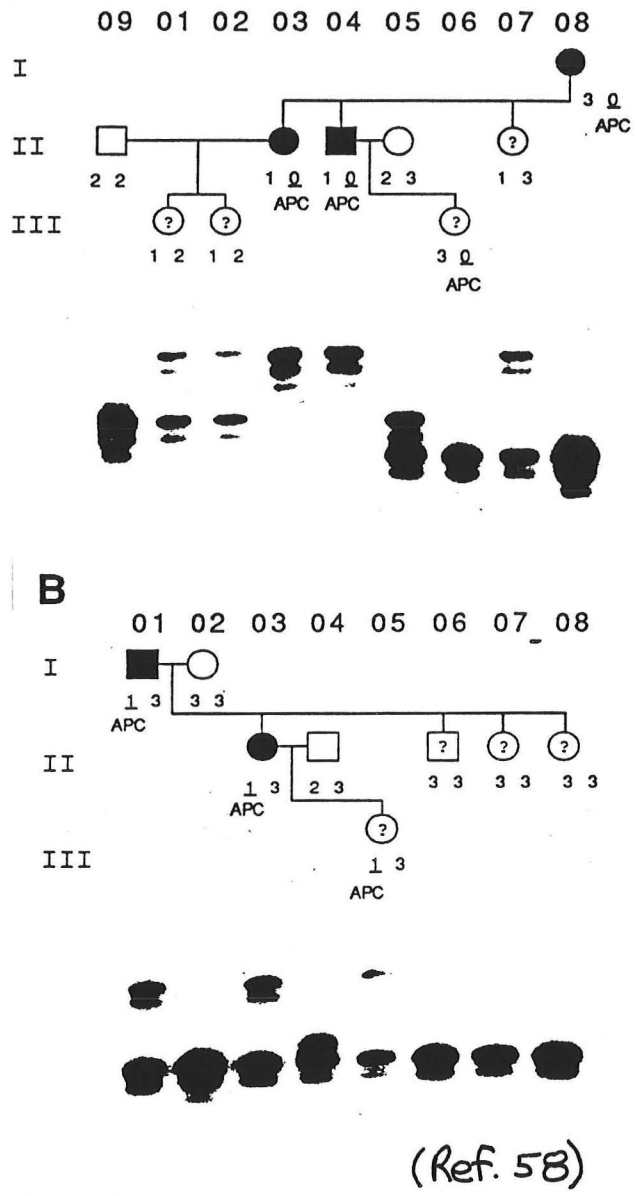


Figure 5 Hybridization patterns of D5S346 genotypes in families APC-12 (*A*) and APC-13 (*B*). The CA-repeat polymorphism reveals a deletion, at the D5S346 locus, that segregates with the disease phenotype in APC-12; the autoradiograph for APC-13 is a typical example of an informative analysis.

This type of testing is offered at the University of Utah DNA Diagnostic Laboratory (telephone number (801) 581-8334). Carrier status can be accurately determined, permitting colonoscopic surveillance to be focused upon affected subjects. Moreover, the penetrance by age 40 is close to 100%. With this clear understanding of the natural history, it is theoretically possible to reduce CRC mortality to background levels in FAP kindreds, while also sparing unaffected family members the discomforts and costs of frequent colonoscopy. Practical problems, including nonpaternity, failure to ascertain minimal phenotypes and the appearance of new germline mutations will continue to complicate presymptomatic diagnosis of FAP (58).

5. HNPCC -- HUNT FOR THE GENE

When familiality strongly suggests a genetic basis for a disease like HNPCC, the usual approach to gene identification begins with linkage analysis which can map the gene to within a few million nucleotides. This is done by asking what genetic markers of known map location travel with the disease as it segregates in families. Unfortunately, problems like incomplete penetrance, linkage heterogeneity and lack of informative genetic markers can frustrate a direct, unbiased search for linkage. On the other hand, it is comparatively easy to exclude known mapped genes as candidates responsible for a genetic disease.

According to the Knudsen paradigm (59), the simplest hypothesis to explain HNPCC is a germline inactivating mutation in one growth-restraining (tumor suppressor) gene. Thus, as each candidate tumor suppressor gene was identified by analysis of FAP and common (sporadic) tumors, these genes were examined for linkage to HNPCC in various kindreds. These analyses were uniformly negative; neither *APC, P53,* nor *DCC* were linked to CRC in these kindreds (60,61).

Not having any luck with known genes, two collaborating groups of cancer geneticists, headed by Vogelstein in Baltimore and de la Chapelle in Helsinki, went to work on 2 large HNPCC families, one from North America and another from New Zealand. These families were large enough to establish linkage with acceptable probability if only the right markers were available. They checked for linkage of neoplasia to 250 DNA markers without success. With about 100 family members to check, that's a lot of work. Although frustrated, they were convinced that the genetic hypothesis was correct by the appearance of CRC in a 27 year old family member (61). They then took advantage of a set of highly polymorphic DNA markers, recently available from James Weber and the French workers, who are the world's leading authorities on human genetic polymorphisms (62, 64). These markers, called microsatellites, are short tandem repeats of nucleotides, for example $(C\text{-}A)_n$, interspersed between unique sequences (63) **(Figure 6)**.

FORWARD PRIMER---->

```
      GGGT ATCTGGGCTC TGGGGT
      1120        1130        1140        1150        1160        1170        1180
TTCAAAGGGT ATCTGGGCTC TGGGGTGATT CCCATTGGCC TGTTCCTCCC TTATTTCCCT CATTCATTCA
AAGTTTCCCA TCGACCCGAG ACCCCACTAA GGGTAACCGG ACAAGGAGGG AATAAAGGGA GTAAGTAAGT

      1190        1200        1210        1220        1230        1240        1250
TTCATTCATT CATTCATTCA TTCATTCACC ATGGAGTCTG TGTTCCCTGT GACCTGCACT CGGAAGCCCT
AAGTAAGTAA GTAAGTAAGT AAGTAAGTGG TACCTCAGAC ACAAGGGACA CTGGACGTGA GCCTTCGGGA

                                  CCTCAGAC ACAAGGGACA CTGG
                                  <-------REVERSE PRIMER
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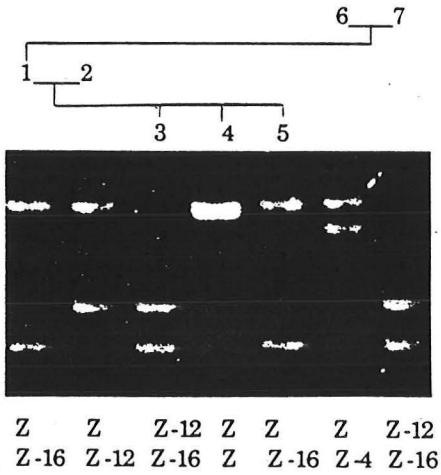
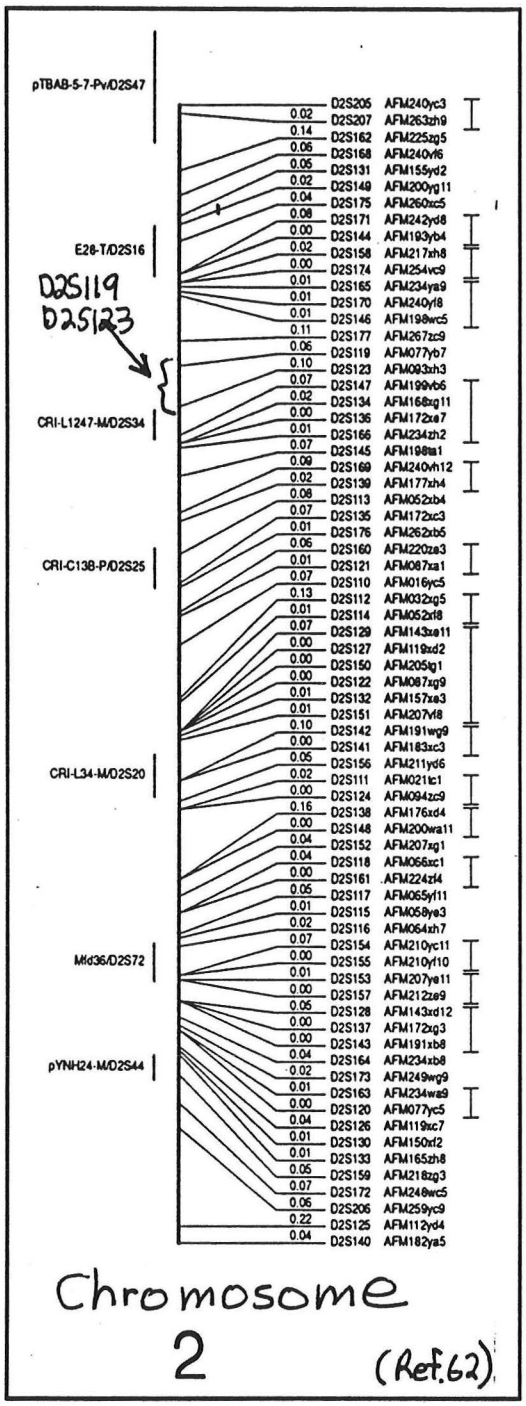
FIG 6



Primer sequences are shown for the CATT tetranucleotide repeat in the tyrosine hydroxylase (*TH*) gene on human chromosome 11p15.5. The database sequence gives an allele length of 108 bp (called here Z–16, 16 bp smaller than Z, the commonest allele) comprising the repeat $(CATT)_9$, forward primer and sequence 5' of the repeat, reverse primer and sequence 3' of the repeat. The respective lengths of these segments are 36 bp + 54 bp + 28 bp = 108 bp. The primers amplify five alleles (Z, Z–4, Z–8, Z–12, Z–16) differing in length by multiples of 4 bp. Four of the five alleles for this locus are shown here segregating in a three generation family. The alleles are resolved using a nondenaturing 10% acrylamide gel (200 mm × 200 mm × 0.75 mm) stained with ethidium bromide. PCR conditions are as described in Ref. 27; loading involves 4 µl of a 25 µl reaction volume from a 96-well microtitre plate, after 30 amplification cycles on a LEP Prem III thermocycler. The alleles of each individual are described beneath each gel lane. Paternal grandfather (individual 6) has *TH* alleles Z and Z–4 and paternal grandmother (individual 7) has Z–12 and Z–16. The grandparents contribute alleles Z and Z–16 to individual 1, who is the father of the three sibs (individuals 3, 4 and 5). Their mother, individual number 2, has alleles Z and Z–12. Sibling 3 inherits the maternal Z–12 and paternal Z–16, sibling 4 is homozygous for Z and sibling 5 has alleles Z (maternal) and Z–16 (paternal).

As reviewed recently in Grand Rounds by Rody Cox (February 11, 1993), these are very powerful tools in linkage analysis for two reasons. First, any given locus is highly polymorphic for n, the repeat number, which is normally inherited by Mendelian rules. This means that heterozygosity is very high, and thus each marker is likely to be informative in any given individual. Second, these markers are spaced relatively uniformly throughout the genome. For example, the $(C\text{-}A)_n$ microsatellites are found on average once every 3×10^4 nucleotides (62,63). Thus, one is likely to be near or within every gene, and a total of about 10^5 such markers are thought to be scattered through the genome. Weissenbach's microsatellites on chromosome 2 are shown in **Figure 7**



(62). After checking about 100 microsatellite markers for linkage, the Baltimore/Helsinki team's hard work finally paid off. Tight linkage to adjacent markers D2S123 and D2S119, found on chromosome 2p15-16, was noted in each family (64) (**Table 6**).

Table 6. Linkage Between DNA Markers and HNPCC in Families C and J

Marker	Family	Maximum Lod Score	Recombination Fraction
D2S119	C	0.64	0.12
D2S119	J	3.43	0.05
D2S123	C	5.24	0.00
D1S123	J	0.83	0.00

From Reference 64. For these calculations, only patients with colon or endometrial carcinoma were considered affected (high-stringency criteria).

For the initiated, lod scores favoring linkage of HNPCC to one of these markers exceeded 3 at θ = 0-0.08, and recombination with flanking markers was observed in both families. Remarkably, linkage of HNPCC to this locus had not been suspected from any previous clinical, cytogenetic or molecular observations (13). This work is a striking example of the power that microsatellite markers bring to linkage analysis. Given the relatively tight linkage, powerful positional cloning strategies will probably succeed in isolating the relevant gene within a year. It already has a name: *FCC*, for familial colon cancer (61).

A key feature of these studies is that linkage to the same locus was found in both families (64). Thus it is likely that *FCC* alleles will be common determinants of HNPCC. Further addressing the issue of heterogeneity, in

14 other HNPCC kindreds analyzed, linkage to chromosome 2p15-16 was formally excluded in 3 (lod score < -2.0) (65). In the other 11, a spectrum of observed linkage probabilities suggested that in about about half the kindreds, HNPCC might be linked to this locus. Problems of undocumented, deceased or young family members described earlier often frustrated more definitive analysis. However, the studies did reveal clear evidence of linkage heterogeneity. As suspected from variability of clinical features, and unlike FAP, HNPCC is not going to result from mutations of a single gene.

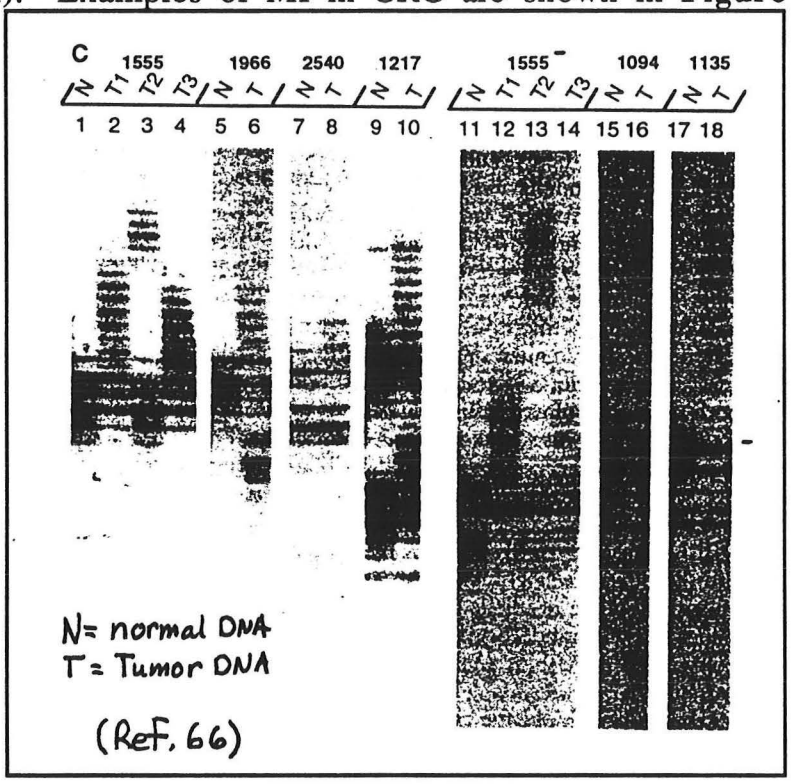
6. HNPCC -- A STUNNING PHENOTYPE IN THE TUMOR GENOME

As discussed earlier, the first idea to test was that *FCC* is a tumor suppressor gene, like *APC* or *P53*. If so, one would expect to see loss of heterozygosity at chromosome 2p15-16 in at least some HNPCC's and possibly in some sporadic CRC's as well. When the Baltimore/Helsinki workers looked, they found no evidence of allele loss at 2p15-16 in either HNPCC or common (sporadic) CRC (65). What they did find were unexpected and astonishing alterations in microsatellite loci. Groups at the Mayo Clinic (Thibodeau) and California Institute for Biological Research (Perucho) have simultaneously published similar findings in subsets of common (sporadic) CRC (66,67).

As already mentioned, microsatellites are regions of repeated short di-, tri- or tetranucleotide DNA sequence patterns, such as $(C\text{-}A)_n$, $(A\text{-}T)_n$, $(C\text{-}A\text{-}G)_n$, $(C\text{-}A\text{-}T\text{-}T)_n$, where n, the repeat number, is highly variable in the population **(Figure 6)**.

This variability arises because replication, repair or recombination of these sequences is slightly more error-prone than that of other DNA. It is thought that the DNA processing machinery occasionally slips during base pairing, losing its precise register in the midst of the redundant sequence (68). By stuttering forwards or backwards, the enzymes may produce imperfect progeny DNA molecules with greater or fewer repeats than found in the parental sequence. While this mechanism, acting across large populations, may account for the extensive polymorphism characteristic of microsatellites, these errors are still quite rare, occuring at each locus perhaps once every 10^4-10^5 generations. As mentioned earlier, the normal situation is stable inheritance of alleles according to Mendelian principles. Indeed, linkage analysis using microsatellite markers depends upon this stability.

Nonetheless, certain mutations may predispose to local microsatellite exansion or contraction during meiosis, leading to the phenomenon of genetic anticipation in diseases like myotonic dystrophy, fragile-X mental retardation and X-linked spinal muscle atrophy. Mike Brown reviewed this phenomenon in detail in his Grand Rounds on March 11 of this year. These are devastating diseases due to (Pyrimidine-G-C) triplet expansion in <u>one</u> locus, with interference of expression of genes at and perhaps near that locus. Now imagine what might happen if a similar phenomenon affected <u>thousands</u> of microsatellite loci during mitosis. This is what has happened in the majority of HNPCC colon tumors analyzed by the Baltimore/Helsinki groups (65). This tumor phenotype is <u>microsatellite instability</u> (MI). Examples of MI in CRC are shown in **Figure 8.**



Six important findings deserve emphasis (**Table 7**).

Table 7. Microsatellite Instability in Colon Cancer

Instability is somatic; in tumors, not normal cells
Both increases and decreases in repeat number
Many loci, perhaps thousands, are unstable
Found in majority of HNPCC
Found in 13% of common (sporadic) colon
Found in synchronious adenomas and carcinomas

References 65-67.

First, the changes are evident by comparison to the normal genotype of the individual; that is, the instability is tumor-specific. Second, both increases and decreases in microsatellite repeat number are found in the tumor genome. Third, tumors display these changes at multiple microsatellite loci chosen at random, including both di- and trinucleotide repeat microsatellites. Fourth, this genomic phenotype was identified in 11 of 14 HNPCC kindreds analyzed by the Baltimore/Helsinki groups (65). Linkage of the phenotype to chromosome 2p15-16 was not addressed. Fifth, the Baltimore/Helsinki (65), Minnesota (66) and California (67) groups independently have discovered the same phenotype in about 13% of common (sporadic) colon cancers. Sixth, synchronous adenomas and carcinomas in a single sporadic patient all expressed the MI phenotype (67).

To summarize these data, a new phenotypic marker for a major subset of HNPCC has been discovered. This MI+ phenotype may have important implications for neoplastic pathogenesis. This is a new type of genetic instability which can disrupt gene expression anywhere in the genome. Although some inhibitory promoter/enhancer regions could be damaged, most of the altered microsatellites would probably result in no change or in loss of function of adjacent genes. Therefore, the phenotype could be a new mechanism for inactivation of critical tumor suppressor genes, a mechanism not involving allele loss. If so, one might expect to see less allele loss in MI+ tumors than in common (sporadic) CRC. In fact, both the Baltimore/Helsinki and Minnesota groups reported that allele loss was much less common in MI+ than in MI- tumors (65,66) (**Table 8**).

Table 8. Allele Loss and Microsatellite Instability in Common (Sporadic) CRC

(A)

MI Status	Number of Tumors	Fraction of Chromosome Arms with Allele Loss
No MI	40	0.254 ± 0.142
MI	6	1.039 ± 0.034

Reference 65. The average ± SD of the fraction of chromosome arms with allele loss is shown.

(B)

MI Status	LOH	No LOH
No MI	146	66
MI	2	28

Reference 66. The number of tumors in each category is shown.

Thus, in individual CRC's, you see either massive allele loss or massive MI, but not both. Perhaps both together would be lethal. This segregation suggests that the two phenomena may be independent rather than complementary mechanisms of gene inactivation in the pathogenesis of CRC. Common to both phenomena is a major breakdown in maintenance of genetic stability. Since these phenomena are probably fundamental mechanisms of tumor progression, their root causes now must be understood. Surely the locus on chromosome 2p15-16 will be an important clue to the cause of MI. Perhaps the mutant gene will be an altered DNA replication or repair enzyme.

Regarding other types of genetic lesions commonly observed in many malignancies, an hypothesis has emerged that loss of P53 function contributes to fixation of damage. Again, I refer you to John Minna's recent Grand Rounds which reviewed this idea (April 22, 1993). Normally, P53 is thought to stop genomically damaged cells from progressing through their division cycle. If the damage cannot be repaired, then P53 activates programmed cell death (apoptosis). Without these functions, genetic damage is magnified and fixed by the processes of DNA replication and mitosis (50-53). Since loss of P53 function and allele loss go together in most common (sporadic) CRC's, one wonders whether P53 loss and MI might <u>not</u> go together. If P53 loss leads to widespread allele loss, we would not expect tumors without allele loss to show P53 inactivation. Right now the preliminary data seem to be in some conflict, although the numbers are difficult to analyze because important information was not presented **(Table 9)**.

Table 9. Loss of P53 Function in CRC

Investigators (Reference)	Type of Tumor	MI	P53 Mutations
Baltimore/ Helsinki (65)	HNPCC	?*	7/11 (67%)
	Sporadic	?	81/132 (61%)
La Jolla (67)	Sporadic	+	4/15 (27%)†
	Sporadic	-	62/107 (58%)

*Fraction MI+ not stated. Overall, 11/14 (79%) HNPCC were MI+; 6/46 (13%) of sporadic tumors were MI+.
†P = 0.022 by Fisher's exact test.

The Baltimore/Helsinki collaboration analyzed 11 HNPCC tumors and found 7 (64%) with evidence of *P53* mutation, a fraction similar to the 61% found in common (sporadic) CRC (65). The authors do not clearly state whether the HNPCC tumors they analyzed for *P53* mutations had MI, although overall 11 of the 14 HNPCC tumors they tested had the MI+ phenotype. On the other hand, Perucho's group, analyzing tumors not known to be familial, found *P53* mutations in 4 of 15 (26%) MI+ and 62 of 107 (58%) MI- cancers (P = 0.022 by Fisher's exact test) (67). Thus, this is still an open issue. Discrepancies in the frequencies of *RAS* mutations in the two groups of tumors are also apparent. Perhaps the differences are due to analysis of different kinds of MI+ tumors, one set of HNPCC and the other of common (sporadic) origin.

7. SPORADIC TUMORS: A ROLE FOR MICROSATELLITE INSTABILITY?

All three groups report that a significant fraction of common (sporadic) CRC's display MI (65-67). If the Minnesota series is confined to "significant" as opposed to "minor" MI mutations, the frequency of the MI+ trait in the 3 series is remarkably consistent at 12-14% of common (sporadic) CRC. Interestingly, the common (sporadic) MI+ tumors resemble HNPCC cancers in several ways which are consistent between the published series **(Table 10)**.

Table 10. Similiarities Between MI+ HNPCC and MI+ Common (Sporadic) Colon Cancers

Characteristic	MI - Sporadic	MI + Sporadic	HNPCC
Proximal to splenic flexure	62/182 (34%)	33/37 (89%)	64/110(67%)
Dukes' stage A or B	92/202 (45%)	25/30 (83%)	82/110 (74%)
Actuarial survival	60% at 2 years	100% at 2 years	60% at 5 years

Compiled from references 13, 65-67.

Compared to CRC's without MI, common (sporadic) cancers with MI originate proximal to the splenic flexure, are more likely to be Dukes' stage B than C or D, to have a better prognosis, and to be diploid or near-diploid and are less likely to show allele loss. Paradoxically, the California study found that common (sporadic) MI+ cancers tended to be less well-

differentiated than MI- tumors (67). However, it is interesting that despite a favorable outcome, many HNPCC also contain poorly differentiated elements (13).

Given the clinical and biological similarities between hereditary and sporadic MI+ colon cancers, we need to know whether germline mutations are at the root of both or whether a somatic mutation in a colonic epithelial stem cell might be responsible for the MI+ phenotype in the sporadic case. Perhaps both circumstances explain different cases, with low penetrance due to modifying genes or environmental factors accounting for apparent sporadic tumor distribution in some families. Relevant to this issue, in the California study synchronous adenomas and carcinomas in one sporadic patient were found to share the MI phenotype, strongly suggesting a germline mutation in this patient (67). This question has serious implications for relatives of patients with MI colon cancer. Resolution of this question will come in part from identification of the linked gene on chromosome 2p15-16. With the appropriate probes, we can then ask whether the *FCC* gene in nonmalignant tissues is normally expressed in patients with common (sporadic) MI colon cancer. It is important to emphasize that only a minority of common (sporadic) right sided colon cancers display the MI phenotype (35% in the Minnesota series (66)).

8. SUMMARY -- A NEW COLON CANCER SYNDROME

These recent studies have defined a clinicomolecular syndrome of colon cancer which is summarized in **Table 11.**

Table 11. Characteristics of MI+ Colon Cancer

Origin proximal to splenic flexure
Dukes' A, B
Favorable prognosis
Diploid: little allele loss
Poorly differentiated histology

The syndrome probably describes most of HNPCC and about 13% of common (sporadic) colon cancer. It can be suspected clinically from its proximal colonic origin and pathologically from morphologically poorly differentiated tumor with diploid or near-diploid DNA content. It is confirmed by straightforward PCR assays for MI, probes for which are available in the Simmons Cancer Center Family Cancer Laboratory (Dr. Gail Tomlinson, Director). Although fresh tissue is preferable, the MI assays can be done from paraffin blocks. This syndrome has important implications

for understanding pathogenesis. When the causative gene is isolated, it will change our approach to screening and treatment of colon cancer. For the immediate future, what practical approach do we recommend for surveillance of relatives of CRC patients?

9. IMPLICATIONS FOR SURVEILLANCE AND TREATMENT

9.1 HNPCC Kindreds (Table 12)

Table 12. Surveillance of Individuals at Risk for HNPCC

Screen all first-degree relatives of cases
Begin screening at age 25
Full colonoscopy
Surveillance interval:
- Normal Colonoscopy: 2-3 years
- Adenoma: yearly

Surveillance for other carcinomas
- Endometrial
- Ovarian
- Proximal GI/GU tract

Treatment:
- Removal of adenomas
- Subtotal colectomy for carcinoma

Reference 13

Lynch has provided straightforward recommendations for following members of HNPCC kindreds (13). Unlike FAP, genetic identification of the ≅ 50% of family members who are carriers is not yet possible. Thus, beginning at age 25 or 5 years earlier than the youngest family case, all first-degree relatives of probands (parents, siblings, children) should be screened for colon adenomas and carcinomas. The key points to remember are the proximal origin of most of the tumors and the development of extracolonic malignancies in many of the kindreds. Thus, screening must visualize the proximal colon; flexible sigmoidoscopy will not do. Frequency of screening depends on findings at first colonoscopy: if normal, screen every 2-3 years; if adenomas, screen yearly. Adenomas should be removed and carcinomas treated by subtotal colectomy. Depending on family history, screening for endometrial, ovarian, and proximal GU and GI tract carcinomas may be necessary. For women at 50% risk, TAH-BSO should be considered after families are completed. Younger women desiring families should have annual vacuum curettage for evaluation of endometrial histology. Once the relevant genes are identified, genetic identification of non-carriers will obviate a lot of this expensive and uncomfortable screening. Remember that HNPCC is clinically and

genetically heterogeneous; thus the need for empiric screening in many families will be with us for a long time.

9.2 Common (Sporadic) CRC **(Table 13)**

Table 13. Surveillance of First-Degree Relatives of Common (Sporadic) CRC Cases

Begin age 40
Screening flexible sigmoidoscopy
Full colonoscopy if adenoma found
Frequency:
Normal sigmoidoscopy = 3-5 years
Adenoma = Yearly until negative twice
FOBT annually

Since first-degree relatives of common (sporadic) CRC patients have a ≅ 2-fold increased risk of CRC, they should be screened with flexible sigmoidoscopy and annual FOBT beginning around age 40. The finding of adenoma mandates full colonoscopy. If adenoma is not found, flexible sigmoidoscopy can be repeated every 3-5 years. What about family members of patients with proximal cancers that might be of the MI+ phenotype? As I discussed, it is not known what fraction of these kindreds might be segregating predisposition alleles which confer greater than the average ≅ 2-fold increased risk for sporadic CRC. Until the genes are isolated and the family studies done, primary care physicians who counsel first degree relatives should find out all they can about the index cancer patient **(Table 14).**

Table 14. Clues to Germline Colon Cancer Gene

Age <50 years at diagnosis
Synchronous or metachronous cancers
Extracolonic carcinomas

Clinical clues which would mandate more intensive or earlier screening of first-degree relatives include age < 50 yr at diagnosis of cancer, the presence of synchronous or metachronous colon cancers, and the finding of extracolonic carcinomas of the Lynch syndrome II type (endometrium, ovary, proximal GU/GI tract). Finally, recall that minimal or "forme fruste" polyposis syndromes can masquerade as common (sporadic) CRC or as HNPCC (7-12).

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