REX

Laboratory Bulletin

January 2005

UPDATES AND INFORMATION FROM REX PATHOLOGY LABORATORY

Issue Number 99

Gut Check #6: Tests for Hereditary Colorectal Carcinoma

Most cases of colorectal carcinoma (CRC) are sporadic with a peak incidence in the seventh decade. Familial clustering of CRC (with cancers often being detected at much younger age) has long been recognized, but the underlying genetic mechanisms responsible have only recently begun to be determined. At the present time, genetic testing is capable of identifying the molecular basis of carcinoma risk in approximately 3% of all CRC. Nevertheless, this knowledge is already being applied to selected cases to identify patients who might benefit from specific medical or surgical intervention and families requiring increased surveillance. The testing is complex and relatively expensive, necessitating thoughtful selection of patients and the appropriate test strategy for a given patient.

It is helpful to divide hereditary colorectal carcinoma (HCRC) syndromes into those associated with multiple polyps (polyposis) and those which are not (non-polyposis). Within the polyposis category, the polyps are further subdivided by histologic type (adenomatous vs. hamartomatous). While genetic mutations have been identified for selected hamartomatous polyposis syndromes, clinical laboratory testing is not yet readily available for this subset of HCRC.

Hereditary Colorectal Carcinoma Syndromes

Name	Gene Abnormality	Inheritance	Other Clinical Assocation	Test*
Polyposis				
Adenomatous Polyps				
Familial Adenomatous Polyposis (FAP)	APC	AD	Desmoid tumors, other tumors, CHRPE**	FAP MS
Attenuated FAP	APC	AD		FAP MS
MYH-Associated Polyposis	MutYH (MYH)AR		Duodenal polyps, CHRPE	MYH
Gardner syndrome	APC	AD	Osteomas, epidermoid cysts	FAP MS
Turcot syndrome	APC	AD	Medulloblastoma	FAP MS
Hamartomatous polyps				
Peutz-Jeghers syndrome	STK11	AD	Perioral lentigines	Special
Juvenile polyposis	MADH4	AD``	Upper GI (MADH4)	Special
	BMPR1A	AD	Lower GI (BMPR1A)	Special
Cowden syndrome	PTEN	AD	Circumoral papillomas; breast, thyroid CA***	Special
Non-polyposis				
HNPCC (Lynch syndrome)	MLH1,MSH2 MSH6, PMS2	AD	Endometrial, gastric, intestinal, urinary tract and brain CA	HNPCC
Muir-Torre syndrome	MSH2, PMS2	AD	Sebaceous adenoma/carcinoma & visceral CA	HNPCC
Turcot syndrome	MLH1, PMS2	AR	Glioblastoma	HNPCC

* FAP MS = FAP mutation screen (Mayo)
MYH = MYH gene analysis (Mayo)
HNPCC = Hereditary Non-Polyposis Colorectal Cancer screen (Mayo)
Special = Test available only available through special arrangements
** CHRPE = congenital hypertrophy of retinal pigment epithelium
*** CA = cancer

Rex Pathology Associates, P.A.

Stephen V. Chiavetta, MD	(919) 784-3060	Keith V. Nance, MD	(919) 784-3286
Timothy R. Carter, MD	(919) 784-3058	Vincent C. Smith, MD	(919) 784-3056
John D. Benson, MD	(919) 784-3059	Rhonda Humphrey, Practice Manager	(919) 784-3063
John P. Sorge, MD	(919) 784-3062		

REX LABORATORY SERVICES
Healthcare for life. Your life.

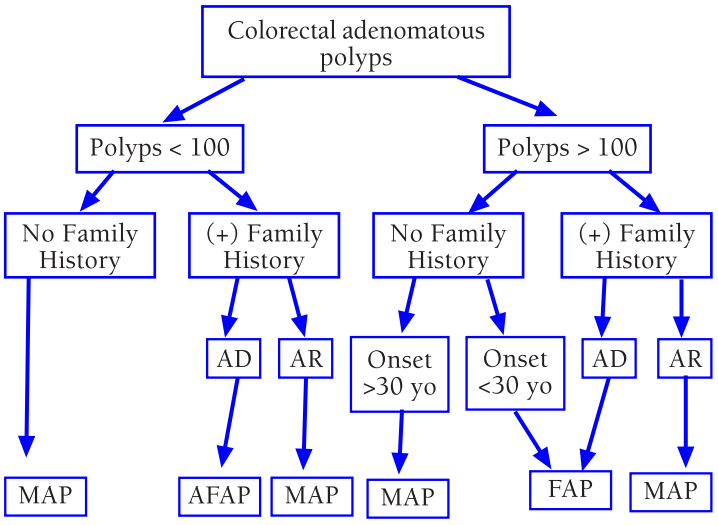
Familial Adenomatous Polyposis (FAP)

FAP is one of the earliest recognized HCRC syndromes. The classical form is characterized by hundreds to thousands of colorectal adenomatous polyps, generally appearing first in the distal colon during adolescence. Extracolonic manifestations include desmoid tumors, gastric polyps, osteomas, thyroid carcinoma, hepatoblastoma and congenital hypertrophy of retinal pigment epithelium (CHRPE). [1] Development of adenocarcinoma in patients with FAP is believed to be inevitable and prophylactic colectomy is recommended. A variant of FAP, referred to as attenuated familial adenomatous polyposis (AFAP), has also been described. AFAP is characterized by fewer (< 100) polyps, which tend to arise in the proximal colon. Both the polyps and subsequent adenocarcinoma have a later onset in patients with AFAP, when compared with FAP. Extracolonic manifestations are not as frequent. Both FAP and AFAP have an autosomal dominant mode of inheritance. The molecular basis for these syndromes involves the adenomatous polyposis coli (APC) tumor suppressor gene. APC mutations may be inherited or develop spontaneously. APC mutations produce defective proteins, which are not as effective in suppressing polyp/tumor growth. Other types of abnormalities in APC may also be observed, and complete evaluation may be rather complex.

MYH-Associated Polyposis (MAP)

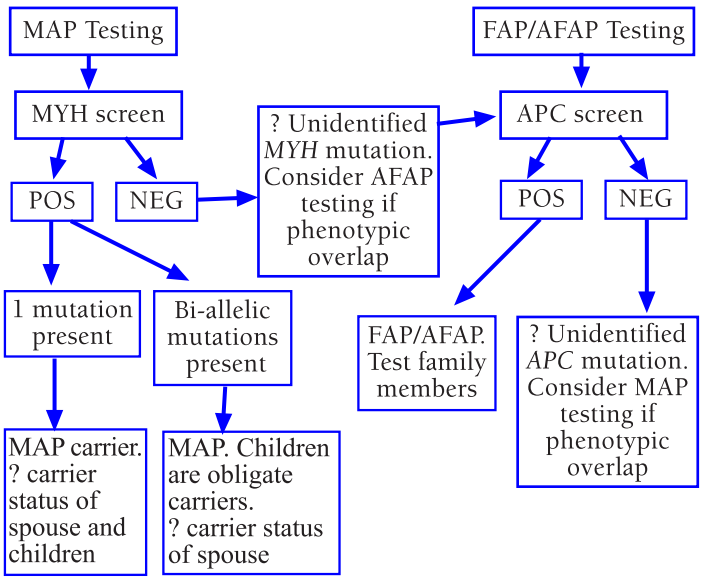
MAP is a recently discovered polyposis, characterized by an autosomal recessive mode of inheritance, but sharing phenotypic features with FAP and AFAP. Most patients have between 10 – 100 polyps, although cases with < 10 or > 100 have been identified. Age of onset is generally later than with FAP. Extracolonic manifestations include duodenal polyps and CHRPE. [1] Mutations in the *MutYH* (*MYH*) gene (part of the base excision repair pathway) are responsible for the polyposis syndrome and risk of CRC. While two specific mutations (Y165 and G382D) account for 85% of recognized Caucasian cases, other mutations have been identified in other ethnic groups. As a result, screening strategies need to consider the ethnicity of the proband, in addition to the number of polyps, family history, and extracolonic manifestations. As there may be overlap in the phenotypic profile of patients with FAP, AFAP and MAP; some patients may require both FAP MS and MYH. As MYH testing is less expensive (particularly for Caucasians), it is recommended as the initial test for cases with phenotypic ambiguity.[1]

Figure 1 Adenomatous Polyposis Test Selection[1]



[1] modified from Baudhuin and Thibodeau
AD = autosomal dominant (vertical) transmission
AR = autosomal recessive (non-vertical) transmission

Figure 2 Adenomatous Polyposis Tests[2]



[1] modified from Baudhuin and Thibodeau

Hereditary Non-Polyposis Colorectal Carcinoma (HNPCC)

HNPCC is the prototype of the non-polyposis HCRC. While originally described in the late 19th century, Henry T. Lynch is credited for reporting detailed clinical manifestations of this disorder (hence, the eponym Lynch syndrome).[2] An autosomal dominant disorder characterized by development of CRC in the fifth decade or earlier, HNPCC may be associated with extracolonic cancers arising in the endometrium, stomach, ovary, small intestine, kidney, ureter, or brain. Colon carcinomas are often proximal and have an "undifferentiated", "medullary" or signet ring histology. A pronounced lymphocytic "host response" has also been reported.[2] Paradoxically, despite the "high grade histology", patients with such tumors often demonstrate better survival than those with more conventional colonic adenocarcinomas. The majority of patients with HNPCC have ***germline*** mutations in one of several genes involved with DNA mismatch repair (MLH1, MSH2, MSH6, PMS2, or MSH3). Defective mismatch repair (MMR) is also associated with microsatellite instability (expansions or contractions in tandem repeats of microsatellite loci in the DNA), a property that is exploited in laboratory evaluation for HNPCC. Neither defective mismatch repair nor

microsatellite instability (MSI) is unique to HNPCC, and both may be found in up to 20% of sporadic CRC. However, sporadic cases are due to ***somatic*** (cf. germline) mutations (generally to MLH1). Determining which individuals should be considered for HNPCC screening requires a detailed family history, as phenotypic clues are few. The original "Amsterdam criteria" are currently considered too restrictive, and have largely been supplanted by the three criteria/guidelines in the table below.

Clinical Diagnosis of HNPCC[1]

Amsterdam criteria II

1. At least 3 relatives with HNPCC-associated cancer (see text)
2. One should be a first-degree relative of the other two
3. At least 2 successive generations should be affected
4. At least 1 should be diagnosed before age 50
5. Tumors should be verified by pathologic examination

Bethesda criteria

1. Individuals with cancer in families meeting the Amsterdam criteria
2. Individuals with 2 HNPCC-related cancers including synchronous and metachronous CRC or associated extracolonic cancers.
3. Individuals with CRC, and a first-degree relative with CRC and/or HNPCC-related extra-colonic cancer and/or a colorectal adenoma; one of the cancers diagnosed at age < 45, the adenoma at age < 40
4. Individuals with CRC or endometrial CA diagnosed at age < 45
5. Individuals with right-sided CRC showing undifferentiated or signet ring histopathology
6. Individuals with adenomas diagnosed at age < 40

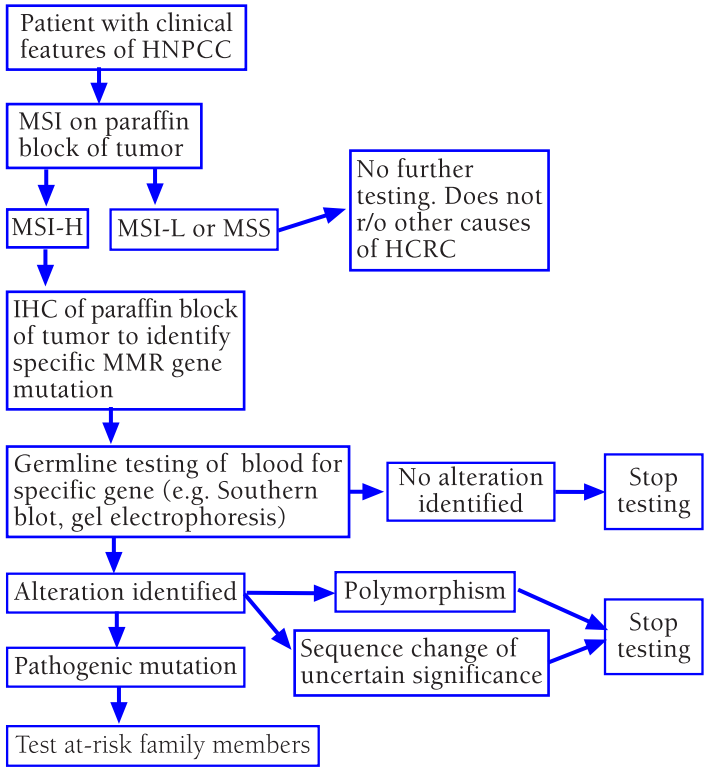
Revised Bethesda guidelines

1. CRC diagnosed in individual at age <50
2. Presence of synchronous, metachronous CRC or other HNPCC-associated cancer (see text) regardless of age
3. CRC with characteristic histology (see text) at age < 60
4. CRC in one or more first-degree relatives with HNPCC-associated cancer, with one of the cancers being diagnosed at age < 50
5. CRC diagnosed in two or more first- or second-degree relatives with HNPCC-associated cancer, regardless of age

[1] modified from Baudhuin & Thibodeau

Once a patient with clinical manifestations suggestive of HNPCC has been identified, testing begins using a paraffin block of carcinoma tissue looking for evidence of MSI by polymerase chain reaction. High frequency (> 30%) microsatellite instability (MSI-H) has a high degree of association with germline MMR gene mutations. Low frequency (< 30%) microsatellite instability (MSI-L) or microsatellite stable (MSS) cases are not associated with MMR gene mutations. If MSI-H is identified, further evaluation for specific mismatch repair gene mutations is performed by immunohistochemistry (IHC). Based on the findings, specific testing for germline MMR mutations can be performed on the patient's blood (Figure 3).

Figure 3 HNPCC Testing [1]



[1] modified from Baudhin & Thibodeau
MSI-H = high frequency microsatellite instability
MSI-L = low frequency microsatellite instability
MSS = microsatellite stability
MMR = DNA mismatch repair genes

HCRC Testing at Rex

All requests for HCRC testing at Rex are referred to Mayo Medical Laboratories. The flowcharts illustrated in figures 2 and 3 reflect the testing algorithms used at Mayo.[1] An *Inherited Cancer Syndromes Patient Information Sheet* must be completed by the ordering physician prior to submission of a specimen. These forms are available from the Reference Desk in the Laboratory, or may be downloaded from the internet by someone having access to the Mayo Medical Laboratory Mayo Link (https://www.mmllink.com/drchart.asp). The value and specificity of the interpretive report is enhanced by accurate clinical data and a detailed family history. ***Genetic counseling prior to testing and after results are available are critical to assure that the findings are put into proper clinical context and that the limitations of the laboratory testing are understood.*** For HNPCC testing, Rex pathologists will be happy to assist in selecting an appropriate paraffin block of carcinoma for MSI and IHC screening for HNPCC. Such screening is not recommended on blocks from adenomatous polyps, as the results are generally non-informative.[3] The screening tests available from Mayo are presented on the next page.

HCRC Screening Tests (Mayo Medical Laboratory)

Test	Turnaround Time (days)	Specimen	Charge($)	Method(s)
FAP MS	14-28	Blood	834*	GE, DNA sequencing, PCR
MYH gene	4-10	Blood	266	PCR
HNPCC screen	14-21	Paraffin Block	368	PCR, IHC
MLH1 screen	14-21	Blood	505	PCR, GE, SB
MSH2 screen	14-21	Blood	437	GE

FAP MS = Familial Adenomatous Polyposis Mutation Screen
MYH = *MYH* gene analysis
GE = gel electrophoresis
PCR = polymerase chain reaction
IHC = immunohistochemical staining
SB = Southern blot
* Additional charges from $173-319 if PCR or DNA sequencing is needed

Separate, but related, tests are used for family members who are undergoing follow-up testing for a ***known*** mutation after one has been identified in the proband by a screening test. It is therefore important to complete the *Inherited Cancer Syndromes Patient Information Sheet* accurately.

John D. Benson, MD

References

1. *Baudhuin LM & Thibodeau SN. Hereditary colorectal cancer. Diagnostic strategies for the clinical laboratory. Clin Lab News July 2004, p. 1418.*
2. *Lynch HT, Smyrk T. Hereditary nonpolyposis colorectal cancer (Lynch syndrome): an updated review. Cancer 1996;78:1149-1167. (A thorough review of the genetic, medical and surgical aspects of all types of HCRC.)*
3. *Burgart, L. Mayo Clinic Dept. of Pathology. Personal communication (12/16/2004). Adenomas are suboptimal due to small amount of DNA and significant stromal (e.g. lymphocyte) contamination. Unusual for adenomas, even in the setting of defective MMR, to have developed significant MSI. Sensitivity of IHC for MMR is also lower in adenomas than carcinomas.*
4. *National Center for Biotechnology Information (National Library of Medicine & National Institutes of Health). http://www.ncbi.nlm.nih.gov/. Good reference for genetic abnormalities in disease.*
5. *Genetics Home Reference (National Library of Medicine & National Institutes of Health) http://ghr.nlm.nih.gov. Good reference for genetic abnormalities in disease.*
6. *About Gene Tests (National Institutes of Health). http://www.genetests.org. Good reference for genetic abnormalities in disease.*

Reference Desk Medical Technologists - Alison Breher, Sue Arwood, Jennifer Deloatch and Renee' Griffin.

Erratum

Erratum #1

Thanks to alert reader Ed Brown, MD (first among several) for discovering a typographical error in Table 2 from last month's *Change in Syphilis Reference Testing* article. The correct table is presented below with the corrected entry highlighted in yellow.

Table 2: Interpretive results for the syphilis panel (Adapted from Mayo Reference Services New Test Announcement November 2004)

Result	IgM	IgG	RPR*
Active or recently treated syphilis	+	+	+
Active or recently treated syphilis	+	+	-
Acute (primary) syphilis	+	-	+
Acute (primary) syphilis	+	-	-
Active or recently treated syphilis	-	+	+
Past, successfully treated, or latent syphilis **	-	+	-
No evidence of active syphilis***	-	-	Not performed

* The RPR is a reflex test for a positive syphilis IgG or IgM. RPR may be useful for determining the current disease status and response to therapy. RPR titers should decrease with successful treatment over time.
** Infants <6 months with IgG or positive RPR, without IgM have probable maternal antibody.
***Severely immunocompromised patients with active syphilis can test negative. Very early primary syphilis and successfully treated (>10 years ago) can also have negative tests. Response to treatment may be indicated by a decrease in RPR titers or the reversion of a positive to negative IgM result.
Positive and equivocal IgM results occurred in patients with the following conditions:
Cardiolipin IgM antibodies (1 of 10 positive)
Anti-ANA, anti-DNA antibodies (3 of 10 positive, 2 of 10 equivocal)
EBV VCA IgM antibodies (2 of 5 positive, 1 of 5 equivocal)
Borrelia IgM antibodies (1 of 10 positive, 1 of 10 equivocal)
CMV IgG antibodies (2 of 5 positive)
Parvovirus B19 IgM antibodies (1 of 5 positive)
Rheumatoid factor IgM (0 of 10 positive)

Erratum #2

Thanks to alert reader Mark Yoffe, MD for spotting the typographical error in the calculations presented in last month's *Abnormal Bleeding following a Broken Hip* case review.

"For a patient with a plasma volume of 2,450 ml., approximately 980 units of factor XI are necessary to administer to raise the plasma level to 40% (0.4 x 2,450 = *1,960*)."

Correction: ..... (0.4 x 2,450 = *980*).

Rex Healthcare Laboratory (919) 784-3040. Telephone extensions are: Pathologists' Direct Line (3063), Sharon Logue (Lab Director 2400), Robin Ivosic (Customer Service and Outreach Manager 3053), Elaine Patterson (Core Lab, Blood Bank and Microbiology Manager 3054), Jackie Okoth (2nd and 3rd Shift Manager 4248), Diane Young (Anatomic Pathology Manager 3888).
Client Response Center (919) 784-6000 (phone) (919) 784-6299 (fax)