Hereditary Colon Cancer Syndromes

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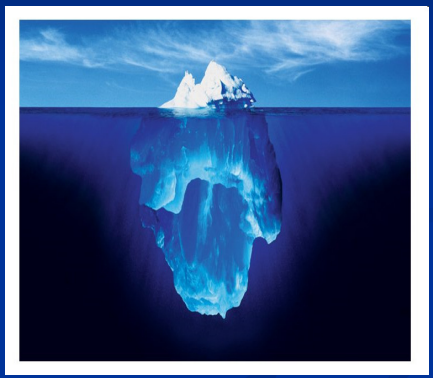
Introduction: Colon Cancer

- Globally, colon cancer is the third most common cancer diagnosed in males and the second in females.
- 1.2 million new cases and 608,700 deaths worldwide in 2008.
- In the U.S., there are about 143,460 new cases annually with 51,690 deaths (accounts for 9% of all cancer deaths).
- 25% of colon cancer is associated with a FH.
- 3-5% of colon cancer is associated with discrete genetic syndromes (Mendelian).

Introduction: Hereditary Colon Cancer

- Suspect large number of undiagnosed cases due to variable penetrance and subtleties in presentation.
- Large number of colon cancer cases in general with hereditary syndromes being relatively common so this is an important issue (0.05 x 143,460=7173).
- Interest developed out of difficulty finding resources to help care for this patient group.



Colon Cancer Syndromes

- HNPCC (Hereditary Non-Polyposis Colo-Rectal Cancer)
- FAP (Familial Adenomatous Polyposis)
- Attenuated FAP
- MYH Polyposis Syndrome
- Suspect a syndrome if see colon cancer or polyps at young age, large number of polyps, distinctive extra-colonic cancers (uterus, small bowel, renal etc…) and a strong family history.

Genetic testing: General Principles

- Specific gene tests are available to aid in diagnosis of the syndrome.
- Once a genetic mutation is identified, family members can be screened and if they test negative, there is no need for intensive cancer screening.
- The absence of a mutation during testing of the **proband** does not rule out a syndrome as we have not identified all of the mutations associated with colon cancer syndromes.
- The absence of a mutation is a true "negative" result only when a clinically affected family member tests positive (the mutation has been clearly identified).

Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndrome)

- Accounts for 1-2% of colon cancer.
- Autosomal Dominant with 80% lifetime risk of colon cancer.
- Average age at colon cancer diagnosis is 45 (? 60's).
- Often relatively few adenomas (often less than 10) and often present in the right colon.
- Often have mucinous or signet ring cell features and a dense lymphocytic infiltrate.
- Uterus is most common site outside of colon.

Cancer risk by location-NCCN (National Comprehensive Cancer Network)

Cancer	General pop risk by age 70	Risk HNPCC MLH1/MSH2	Mean age onset (HNPCC)
Colon	5.5%	40-80%	44-61 years
Endometrium	2.7%	25-60%	48-62 years
Stomach	< 1%	1-13%	56 years
Ovary	1.6%	4-24%	42.5 years
Hepatobiliary	< 1%	1.4-4%	50-57 years
Urinary tract	< 1%	1-4%	54-60 years
Small bowel	< 1%	3-6%	47-49 years
Brain/CNS	< 1%	1-3%	50 years
Pancreas	<1%	1-6%	Not reported

NCCN (National Comprehensive Cancer Network) Guidelines, version 2.2013

Genetics of HNPCC

- MMR proteins (mismatch repair proteins) normally correct errors in DNA replication.
- Germline mutations in MMR genes leads to dysfunctional MMR proteins that in turns leads to accumulation of mutations in microsatellite sequences (short, repetitive DNA sequences)---results in microsatellite instability→uncontrolled cell growth.
- 90% of HNPCC associated with mutations of the MMR genes MSH2 or MLH1.
- MSH6 and PMS2 (attenuated-lower penetrance).

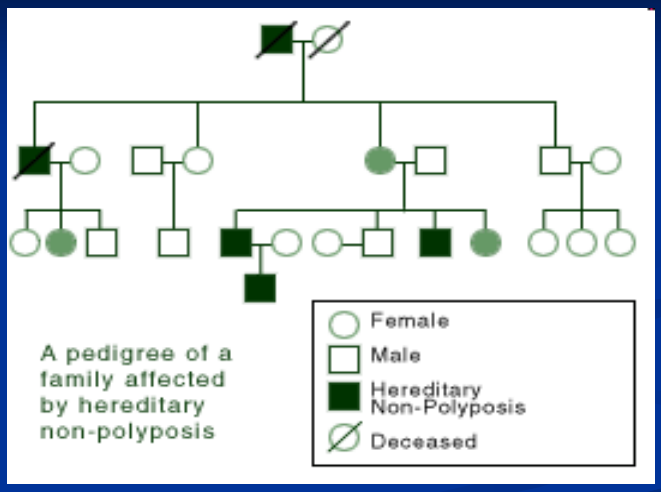
Amsterdam 1 Criteria 1990

- 3 relatives with colon cancer, 1 a first-degree relative of the other 2
- Cases span at least 2 generations
- At least one colon cancer case is diagnosed before age 50

Amsterdam 2 Criteria

As above, but 3 relatives with an HNPCC-associated cancer (colon, endometrial, small bowel, ureter, renal pelvis)

*******The original criteria are highly specific but lack sensitivity (miss people with the disease) due to the stringent criteria. Hence the development of the Amsterdam 2 and ultimately the Bethesda criteria.



A pedigree of a family affected by hereditary non-polyposis

Revised Bethesda Criteria: 2004

Increased Sensitivity (less stringent criteria to catch everyone)

- CRC diagnosed in individual under age 50 years.
- Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors, regardless of age.
- CRC with specific histology (presence of tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern), in patient before 60 years of age.
- Individual with colon cancer and 1 or more first-degree relatives with an HNPCC-related tumor, with 1 of the cancers being diagnosed under age 50 years.
- Individual with colon cancer and 2 or more first or second-degree relatives with HNPCC- related tumors, regardless of age.

Newer Predictive Models

- Over the past few years, predictive models have been developed to improve on operating characteristics of AC/BC (all are investigational).
- PREMM, MMRpro, Leiden and MMRpredict.
- Utilize and number of variables including age, sex, tumor location, presence of extracolonic tumors, family history of cancer and others to quantitate the likelihood of finding a discrete mutation during germline genetic testing.

- PREMM---web based decision making tool that is user friendly and provides valuable risk based information in identifying MLH1, MSH2 and MSH6 mutations.
- Although the PREMM model can be used in both patients with cancer and unaffected, at risk family members, other approaches are under investigation that focus more on demographic and FH data of unaffected at risk individuals, as opposed to the CRC proband.

HNPCC- MSI (Microsatellite Instability Testing) of Tumor Tissue

- MSI-H suggests Lynch syndrome.
- Exceptions
  - MSI-H can be seen in up to 15% of sporadic colon cancers: epigenetic silencing of MLHI by hypermethlylation of MLHI promotor---association with BRAF mutation (unclear mechanism)
  - MSH6 and PMS2 can have lower rates of microsatellite instability.
- MSI-H---proceed to germline testing (blood test) to identify specific mutation.

MSI Testing

- Testing done on DNA extracted from paraffin-embedded tissue sections ideally containing >50% tumor cells.
- Normal control DNA is derived from adjacent normal mucosa.
- DNA analyzed by PCR and then subjected to size-based electrophoretic separation.
- Compare electrophoretic patterns in tumor vs. normal DNA (look for novel DNA fragments in tumor DNA).
- MSI-H, MSI-L or MSS.

Can MSI testing be done on colon polyps?

- Try to avoid.
- Difficult to obtain enough DNA from polyps to do MSI testing and rarely enough normal tissue to serve as a control.

HNPCC- IHC (Immunohistochemistry)

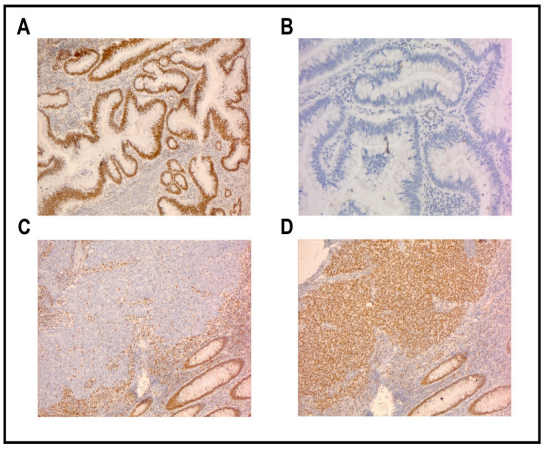
- Test tumor tissue for expression of MMR proteins (MLH1, MSH2, MSH6, PMS2).
- See loss of MMR proteins in Lynch syndrome
- IHC can add extra information compared with MSI alone because MSH6 and PMS2 can have lower rates of MSI that wouldn't prompt genetic testing (false negative if check MSI alone).
- Loss of MMR protein on IHC—proceed to germline testing to identify specific mutation.
- IHC reduces cost of germline testing because one can focus on the missing MMR protein.

IHC examples

Tumor #1
MSH2 mutant

+ MLH1

- MSH2



Tumor #2
MLH1 mutant

- MLH1

+ MSH2

Zavodna *et al. BMC Cancer* 2009 **9**:405

Germline Testing in HNPCC

- Direct sequencing: can miss large deletions, duplications or other genomic rearrangements (can range from 5-30% in some families).
- MLPA (multiplex ligation-dependent probe amplification) can detect what is missed above.
- Variants of unknown clinical significance (VUSs) can be seen in as high as 20-40% of cases (no clear pathogenic mutation).

New Mutations: International Society for GI Hereditary Tumors (INSIGHT meeting 2011)

- Single nucleotide epimutation of MLHI promoter leads to transcriptional silencing of MLHI gene (Rapkins et al, Australia).
- Associated with high levels promoter methylation.
- MSI-H but actual MLH1 gene normal.

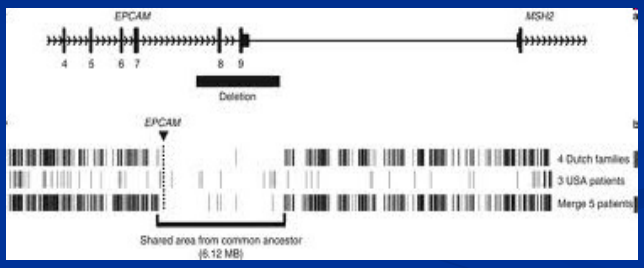
Rapkins et al. University of New South Wales, Australia

New mutations continued

- 3' EPCAM deletions (just upstream from MSH2) lead to epigenetic silencing of MSH2 via hypermethylation of MSH2 promoter (Kuiper et al, Netherlands)
- There may be a lower risk of endometrial cancer in EPCAM deletions unless the deletion is large and extends close to the MSH2 promoter.

Kuiper et al, Netherlands

EPCAM/MSH2



New mutations continued

- Study of 130 CRC patients in New Foundland with a strong family history of colon cancer.
- GALNT12: encodes an enzyme involved in glycosylation and is highly expressed in the colon.
- GALNT12 sequenced in all 130 patients to see if variants in the gene that are associated with low enzyme activity segregate with the disease.
- 4 patients with no other mutations (APC, MLH1 MSH2 etc…) were found to harbor GALNT12 variants supporting a possible association with GALNT12 and hereditary colon cancer. (No control group however)

Michael Woods et al. Memorial University, New Foundland

Principles of Testing

- If identify mutation, test relatives.
- If relatives test negative after mutation is identified, they are average risk.
- If you strongly suspect that someone has HNPCC but gene test is negative, enroll in appropriate high risk screening program (we can not currently detect all mutations associated with HNPCC).
- *Up to 30% of mutations in HNPCC (and other syndromes) can be **de-novo** meaning there is no family history.

Universal Testing of Tumor Tissue

- Testing all patients with CRC (regardless of age or family history) with MSI and/or IHC to decide if germline testing should be performed.
- Greatest sensitivity for detecting HNPCC/LS.
- Can also add information regarding prognosis in sporadic methylation dependent CRC (differences in prognostic and chemotherapeutic sensitivities amongst stage II and III MSI tumors).

- Recent study showed that 38% of cancer centers nationally were using universal testing (more likely in NCI-designated Comprehensive Cancer Centers). *

* Beamer LC et al. J Clin Oncol April 2012

Management

- Surveillance colonoscopy between ages 20 and 25 or 10 years earlier than the age of diagnosis of the youngest case in the family.
- Repeat q1-2 years.
- Annual colonoscopy after 40
- Surveillance has be shown in the literature to decrease mortality in HNPCC/LS
- Study in Netherlands showed a significant decrease in the standard mortality ratio (SMR) from CRC when a national surveillance protocol (after 1990), including colonoscopy, was put in place for patients with LS/HNPCC. *
- Subtotal colectomy with ileorectal anastomosis

* De Jong et al. Gastroenterology, March 2006

Management Continued

- Annual transvaginal ultrasound (uterus/ovaries) and endometrial biopsies (sensitivity and specificity less than ideal). *
- No standard guidelines for other extracolonic tumors- can decide based on family history (periodic renal ultrasound for tumors of uroepithelial tract, periodic endoscopy for gastric cancer etc.).

* Auranen A et al. Acta Obstet Gynecol Scand. May 2011

Surgery in HNPCC

- Studies have shown an increased risk of metachronous CRC in patients who underwent segmental resection as opposed to colectomy. *
- Studies have shown increased life expectancy, particularly in younger patients, in those undergoing extended colectomy. *
- However some patients and surgeons, after careful discussion of risks/benefits opt for segmental resection.
- Regardless of surgery, careful post-op surveillance of remaining colon is critical.

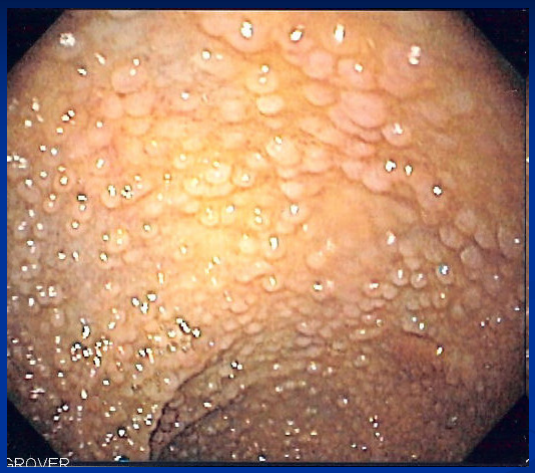
* Natarajan N et al. Dis Colon Rectum 2010.
* Parry S et al. Gut 2011.
* De Vos et al. Gut 2003.

Prophylactic Surgery in HNPCC

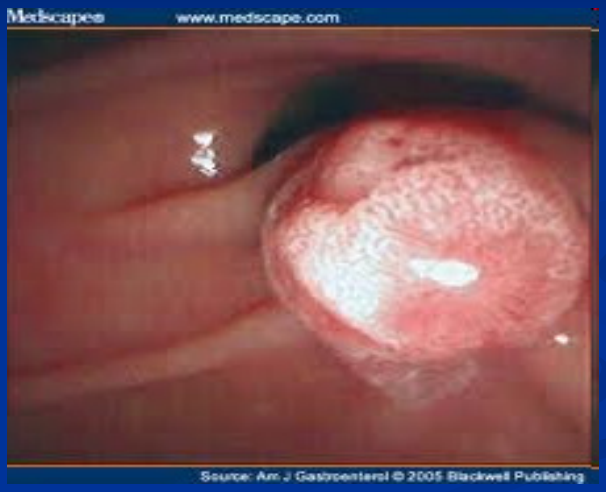
- Although there is a paucity of data and no clear recommendation for this approach, some patients desire prophylactic colectomy (primary prophylaxis) in order to prevent the development of cancer (particularly in young patients or if multiple adenomas or they are reluctant to continue with yearly screening colonoscopies).
- Similarly, some opt for risk reducing prophylactic hysterectomy and bilateral salpingo-oopherectomy.

Familial Adenomatous Polyposis (FAP)

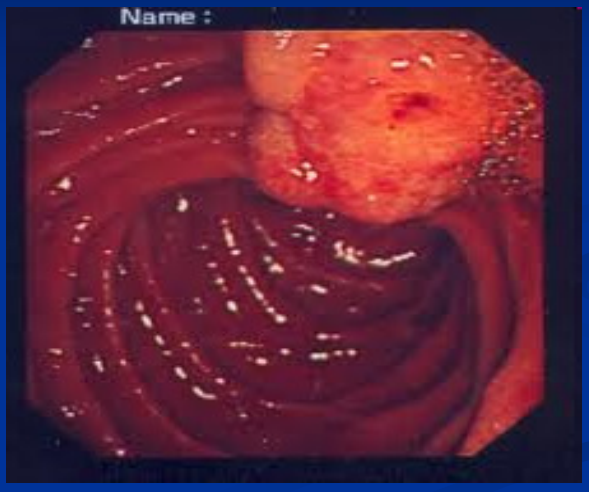
- Multiple colonic adenomas throughout colon developing during teenage years.
- AD, penetrance 100% with colon cancer < age 40.
- Duodenal, peri-ampullary and ampullary adenomas in almost all FAP patients.
- 10% duodenal adenocarcinoma by age 60 (2nd most common cancer in FAP).
- Gastric adenomas/adenocarcinomas, fundic gland polyps.
- Thyroid cancer, congenital hypertrophy of the retinal pigmented epithelium (CHRPE), desmoid tumors, osteomas, medulloblastomas.



Small Ampullary Adenoma



Adenoma with HGD



Desmoids

- Frequent in patients with FAP.
- Often induced by surgery.
- Avoid elective surgery if possible as this can induce desmoids and make future surgery (colectomy) more difficult.
- Prophylactic colectomy is often delayed to try to prevent the development of desmoids.

Lynch, H. NEJM 348;10. 2003

Who develops desmoids? INSIGHT 2011

- Authors studied 559 patients who underwent colectomy for FAP.
- 22 patients (4%) found to have desmoids intraoperatively.
- 27 patients (5%) developed desmoids postoperatively (median 34 months post-op).
- Female gender and 3' APC mutation locations found to be independent predictors for desmoids.
- Type of operation and age were not associated with desmoid development.
- Delay surgery if risk factors for desmoids?

Sinha et al. St. Mark's Hospital, UK

Genetics of FAP

- Mutation of APC gene, a tumor suppresor gene on chromosome 5q21.
- Mutations tend to be in the central region of the APC gene.
- Attenuated FAP mutations are more at the 5' and 3' ends-this leads to a partially functional APC protein.
- Direct DNA sequencing is available to test for FAP.
- 20% of mutations are de-novo with no family history.

Management

- Annual flexible sigmoidoscopy by age 10-12.
- Upper endoscopy + side viewing scope at age 30 or time of colectomy, q1-3 years.
- Annual thyroid ultrasounds.
- Total procto-colectomy with ileo-anal anastomosis (surgery almost always inevitable).
- Some surgeons will leave rectum in place (possible to be removed at a later time)---approach depends on surgeons experience and polyp burden in rectum.
- With either surgical approach, intensive surveillance of ileal pouch or residual rectal mucosa is critical.

Chemoprevention

- Patients with FAP treated with celecoxib 400 mg po bid for 6 months had a 28% decrease in the number of adenomas compared with placebo (1).
- Regression of colonic adenomas occurred in all patients after 6 months of treatment of Sulindac 200 mg po qd (2).
- However after a mean of 48.6 months the number and size of polyps increased in those who received Sulindac.
- In addition, Sulindac did not effect the progression to malignant polyps.

1) Steinbach et al. NEJM 2000; 342:1946-52
2) Tonelli et al.J Surg Onc 2000;74:15-20

Novel Agents: INSIGHT meeting 2011

- Polyunsaturated fatty acids (eicosapentaenoic acid-EPA) studied in APC+ mice.
- APC+ mice given placebo, 2.5% or 5% EPA for 12 weeks.
- EPA 2.5% and 5% suppressed new polyp formation by 71% and 79% respectively.
- EPA 2.5% and 5% reduced polyp load by 82% and 93% respectively.
- At the cellular level, a significant reduction in Cox-2 expression was seen along with high levels of apoptosis.

Fini et al. University of Bologna, Italy

EPA in humans-INSIGHT meeting 2011

- FAP patients with previous colectomy and ileo-rectal anastomosis were randomized to EPA-FFA (2 grams/day) or placebo.
- A tattooed area in the rectum with high polyp burden was followed over time for changes in polyp number and size for 6 months.
- Polyp number decreased by 22% and polyp size decreased by 30%.

West et al. St. Mark's Hospital UK.

Attenuated FAP

- Less than 100 adenomas, often only 10-20, sometimes even less.
- Diagnosis of polyps and cancer delayed 15 years compared with traditional FAP (colon cancer in 50's).
- Colon cancer lifetime risk is 80% or higher so critical to recognize this entity.
- Extraintestinal manifestations similar to FAP including upper tract lesions.

Burt RW.
Gastroenterology. 2003 Nov;125(5):1462-9. Review.

Recommendations for genetic testing

- >10 colonic adenomas on 1 colonoscopy or over time.
- Multiple adenomas at a younger age (< 40) or with family history of colon cancer or extraintestinal manifestations.
- Clinical judgment important in recognizing this condition and recommending testing as different mutations may have different penetrance and hence variable clinical presentations.

AGA technical review on hereditary colorectal cancer and genetic testing.
Giardiello FM, Brensinger JD, Petersen GM.
Gastroenterology. 2001 Jul;121(1):198-213. Review

Rex et al. American Journal of Gastro, 2009 March; 104 (3): 739-750

Management

- Patients need annual colonoscopy starting in late teens.
- Upper tract surveillance the same as FAP.
- Can manage with colonoscopy with polypectomy until polyps become numerous, advanced or difficult to remove or if colon cancer is discovered.
- Subtotal colectomy with ileo-rectal anastamosis as compared with FAP given often minimal rectal involvement in AFAP.

MYH polyposis syndrome

- Autosomal recessive.
- 22-29% of European population with > 10 adenomas have been found to have this condition.
- Risk of colon cancer over lifetime approaches 100%.
- Can resemble AFAP but parents have no history of colon cancer. Can confuse with de-novo AFAP.
- Often do genetic testing if suspect AFAP but APC sequencing is normal.

Conclusions

- HNPCC is the most complicated of the syndromes due to the large number of genes involved.
- FAP is relatively straightforward to diagnose due to obvious phenotype and fact that only one gene is involved.
- AFAP and MYH can be difficult to diagnose so physicians needs a high degree of suspicion.
- A negative family history does not rule out a syndrome as mutations can be de novo.
- Tumors outside of the colon can be the first manifestation of a syndrome.