Genetics of Colon Cancer in Teenagers

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Risk factors for Colon Cancer

Situation	Lifetime Risk of CRC
General Population	6%
Personal history of CRC	15-20%
Inflammatory Bowel Disease	15-40%
Hereditary Nonpolyposis Colon Cancer (HNPCC)	60-80%
Familial Adenomatous Polyposis	>95%
Childhood HD survivor	RR 6.0

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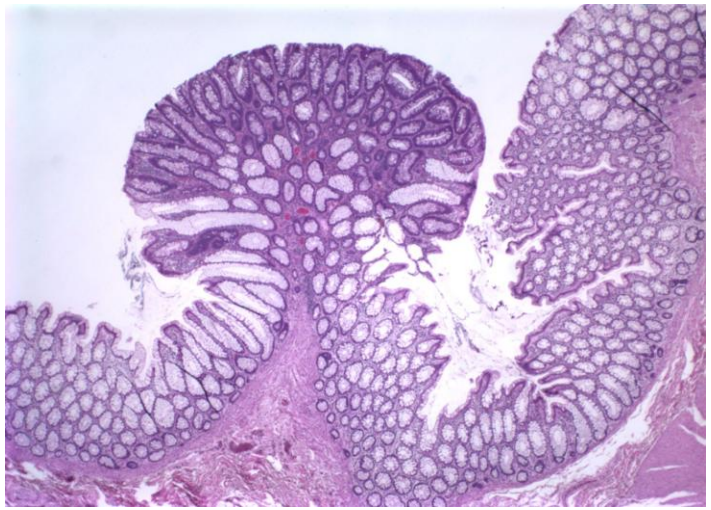
Familial Adenomatous Polyposis (FAP)

- Autosomal Dominant with very high penetrance.
- 15-30% represent new mutation cases and have no family history of disease.
- Adenomatous colonic polyps begin in childhood to adolescence.
- Extracolonic features first noted by Gardner and called Gardner Syndrome. Now lumped together.

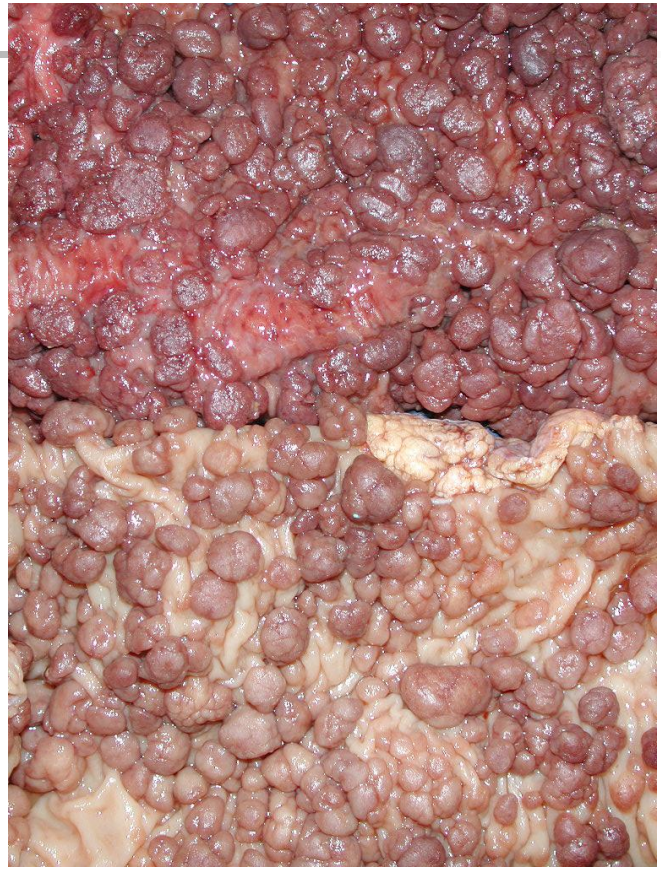
FAP - Extracolonic Manifestations

- Desmoid tumors – often abdominal. Painful and very difficult to treat.
- Osteomas of the jaw, skull, or other bones
- Epidermoid cysts on face or trunk
- Congenital Hypertrophy of the Retinal Pigment Epithelium (CHRPE) – present at birth, asymptomatic but very useful clinically.
- Pediatric hepatoblastoma (~0.5-1% risk)
- Thyroid cancer (1% risk)

Polyposis Associated with FAP



Typical Adenomatous Polyp from colon of a teenager with FAP



Twin A

Twin B

Colons of twin teenage boys who presented with history of rectal bleeding and abdominal pain

Photographs courtesy of M. Finegold, MD – Baylor College of Medicine/Texas Children's Hospital

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Mutations in *APC* cause FAP

- ~ 80% truncating mutations in the APC gene.
  - Originally used protein truncation testing.
  - D-HPLC and now direct sequencing used for testing.
  - Test for deletions (5-10%) if sequencing is negative.
- Some genotype:phenotype correlation.
  - Extracolonic symptoms tend to be associated with mutations in the middle, particularly long exon 15.
  - Attenuated FAP (fewer polyps, later onset of CRC) mutations cluster at the far 5' and 3' ends of the gene.

Genetic Counseling in FAP

- *APC* testing is gold standard for utility of genetic testing.
  - Identifies which family members need surveillance and eventual surgery.
- Guidelines typically recommend testing around age 10 – 12.
  - Some consideration of testing and screening for hepatoblastoma in infants.
  - Practical issues may suggest earlier testing.

Management of APC+ Patients

- Initiate testing with family member with polyposis in order to identify mutation.
- Then test at risk individuals for familial mutation.
- Positive individuals undergo management:
  - Colonoscopy beginning age 10 – 12; continuing every 1-2 years.
  - Colectomy by late teens to early twenties (depending on polyp load or dysplasia).
- Upper GI follow-up by endoscopy for risk of gastric adenomas and duodenal carcinomas.
- Annual thyroid exam

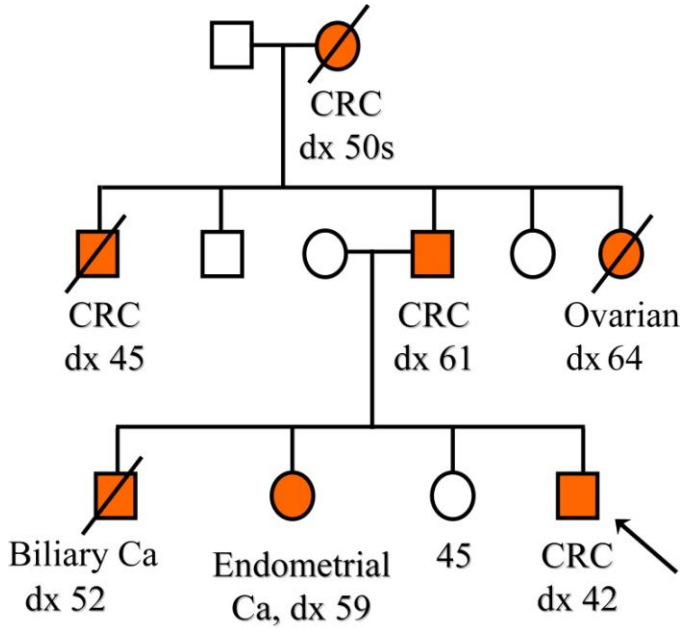
Adolescent FAP (Vasudevan et al, J Ped Surg, 2007)

- Reviewed all cases undergoing colectomy for FAP indications at TCH (<age 18).
- N = 11 (1998-2004). Mean age 13 yrs.
  - 0 invasive carcinoma
  - 3 carcinoma-in-situ or severe dysplasia
  - 9 dysplasia
- In constrast invasive CRC clearly occurs in young adults with FAP.
  - Lack of insurance coverage of young adults in the US often argues for colectomy in late high school ages.

Hereditary Non-Polyposis Colon Cancer (Lynch Syndrome)

- Autosomal Dominant CRC without polyposis.
- ~70% lifetime risk of CRC (often right-sided) and 50-70% Endometrial Ca.
  - Ovarian, biliary tract, ureteral, gliomas also seen in HNPCC families.
  - Common to see individuals with 2 or 3 different primary HNPCC-related tumors.

Family History Criteria for HNPCC



- Amsterdam Criteria (CRC based)
  - Exclude FAP
  - At least 1 CRC < age 50
  - 2 affected generations
  - 3 affected relatives, 2 are 1° relatives of other one
- Bethesda Criteria – proband:
  - CRC < 50
  - CRC + HNPCC assoc Ca
  - 1° relative with 2 HNPCC cancers, one of which < age 50
  - 2 – 2nd degree relatives, one with CRC and one with HNPCC associated cancer

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HNPCC

- Due to heterozygous mutations in one of four mismatch repair (MMR) genes:
  - *MSH2, MLH1, MSH6* and *PMS2.*
- Follows the two-hit hypothesis:
  - Autosomal dominant inheritance of one mutation with loss of the second copy in the tumor cell.
  - This results in the tumor cell being mismatch repair defective even though normal cells still have one working copy and are MMR active.
- In 20% of sporadic CRC both copies of the *MLH1* gene can be silenced by methylation.

HNPCC – Clinical Testing and Screening

- Most protocols suggest performing MIS testing or IHC for MMR proteins in early onset CRC.
  - If MIS+ or IHC shows missing protein (and not methylation of *MLH1*) then do mutation analysis of *MSH2, MLH1* and *MSH6*.
  - Need to do copy number analysis as *MSH2* deletions are common.
  - *PMS2* sequencing and copy number analysis now available.
  - Experience demonstrates that this is rarely done on a consistent basis!!

HNPCC - Surveillance

- Screening with colonoscopy every 1-2 years starting at age 25 clearly decreases mortality in mutation carriers.
  - Prophylactic colectomy only recommended on case-by-case basis.
- Screening for endometrial cancer by endometrial biopsy recommended.
  - Effectiveness less.
- Prophylactic oopherectomy being discussed but not part of guidelines.

AYA CRC and HNPCC (Durno et al, *Gut*, 2005)

- Study looking at familial CRC database for probands diagnosed <age 24 (n=16; 1% of registry).
  - Microsatellite instability was identified in tumors from eight (73%) of 11 evaluated patients.
  - Germline mutations in mismatch repair genes were identified in six of 12 patients, including *MSH2* (n = 3), *MLH1* (n = 2), and *PMS2* (n = 1). One homozygous case. *MSH6* not done.
  - Ten (63%) of 16 families met the Amsterdam criteria for HNPCC.
  - Location - rectum/sigmoid (n = 9), splenic flexure (n = 2), hepatic flexure (n = 3), and caecum (n = 2).
  - 44% (7/16) developed additional malignancies (gastrointestinal (n = 8) and extraintestinal (n = 4)) during follow up (mean 12.8 (SD 12.4) years).

MIN vs CIN Hypothesis

- Microsatellite instability (MIN)
- Relatively diploid genome.
- Oncogenic events due to expansion of repeats in genes like TGFBR1

- Chromosomal Instability (CIN)
- Associated with APC defective pathway
- Aneuploidy
- Large-scale rearrangements in tumors.

Modified from Lengauer …. Vogelstein *Nature, 1996*

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Turcot syndrome. Autosomal dominant or recessive transmission?

Costa OL, Silva DM, Colnago FA, Vieira MS, Musso C.

Dis Colon Rectum. ***1987*** 30(5):391-4.

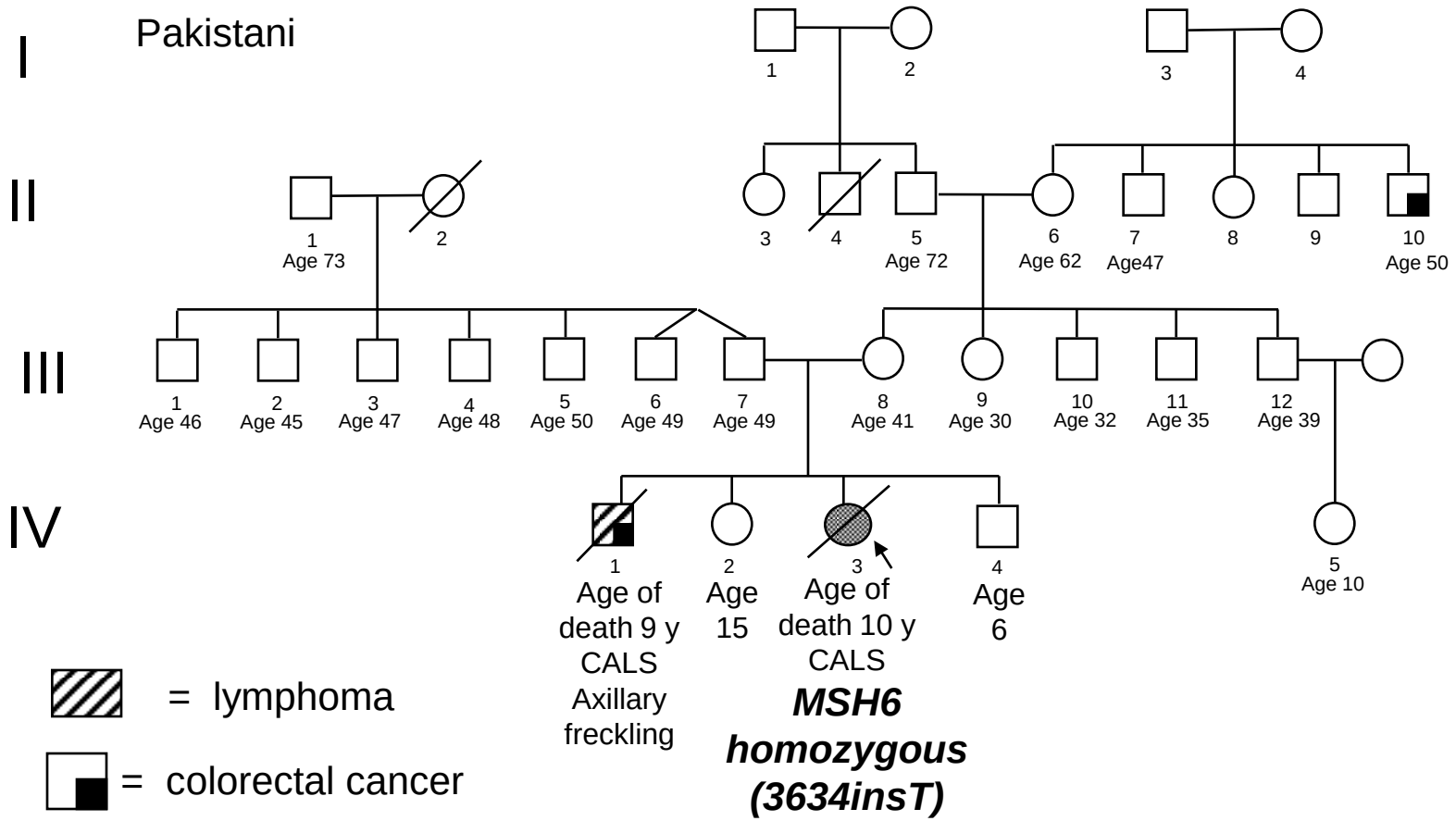
AR Turcot/ Mismatch Repair Deficiency Syndrome (MMR-D)

- Turcot Syndrome – association of brain tumors and colon polyps/cancer in childhood.
- Dominant forms (rarely have adolescent colon cancer):
  - FAP and medulloblastomas
  - HNPCC and glioblastomas
- Autosomal Recessive forms – associated with childhood, adolescent and young adult colon cancers.

Turcot Syndrome

- The eponym Turcot Syndrome often refers to a family with colonic polyposis and brain tumors in childhood.
- However, there has been ongoing controversy about how many distinct disorders underlie Turcot syndrome ranging from:
  - Heterozygous mutations in APC in a child with medulloblastoma and colon polyps
  - Homozygous/compound heterozygous mutations in mismatch repair genes.

Typical MMR-D Presentation



Modified from Hegde, et al.,*Clin Cancer Res,* 2005

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Other MMR-D presentations

- Ten year old Pakistani child presented with bilateral glioblastoma lesions and thoracic T-cell lymphoma:
  - Homozygous for intragenic deletion of *PMS2.*
- Nine year old Hispanic child with glioblastoma multiforme:
  - Sibling previous died with T-cell leukemia and GBM
  - *PMS2 intragenic deletion and missense mutation*
- No significant cancer history in rest of family.

Mismatch Repair Deficiency Syndrome (Scott et al., 2006)

- The MMR-D label clearly conveys a condition resulting from inheriting two inactivating mutations in a mismatch repair gene.
  - Some authors (Wimmer & Etzler, 2008) add constitutional to the name (cMMR-D).
  - Autosomal recessive inheritance with consanguinity frequently described.
  - Now been reported to result from biallelic mutations in *MLH1, MSH2, MSH6, PMS2.*

NF1 phenotype

- It's important to realize that constitutional MMR deficiency results in biallelic somatic mutations in the *NF1* gene leading to features of NF1 (Wang et al, Hum Genet, 2003).
  - Mutations can occur anytime in development and result in apparent "segmental" distribution
  - CALS are often atypical in appearance.
- A subset of MMR-D patients will meet diagnostic criteria for NF1 although they don't have NF1.
  - Complicates genetic evaluation and counseling.

Tumor Presentation

- Spectrum of tumors clearly distinct from those seen in HNPCC
- Table was compiled from families with two truncating alleles in MMR genes as of 2007
- All tumors listed were <u>diagnosed in childhood</u>

Total = 32 individuals; 17 families	
Colorectal	8
Brain Tumors	13
Leukemia/ Lymphoma	13
Other	6

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Hematologic Malignancies

- Leukemias and lymphomas are most commonly T-cell
  - In contrast to predominance of B-cell malignancies in children in general population.
  - Rare AML cases have been reported. In some cases may be 2^{o} to treatment.
- Lymphomas are not seen in HNPCC families.

MIS in Sporadic Leukemia?

- In sporadic cancers, microsatellite instability is rare in primary leukemias:
  - Reported in patients who relapse after treatment for primary leukemia
  - Reported in children who develop a secondary leukemia after treatment for a primary malignancy.
- So it appears that MIS requires external insult to be present in leukemia/lymphomas

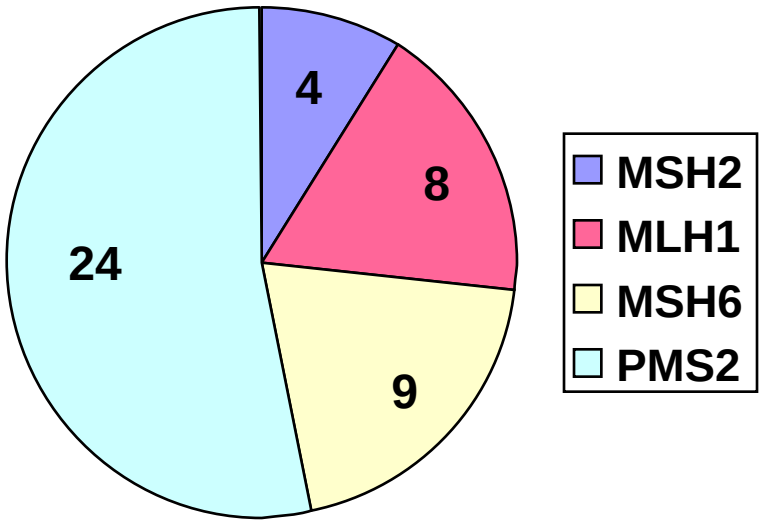
Brain Tumors

- All grades of gliomas reported (including gliosarcomas).
  - High grade (glioblastoma multiforme) frequent.
- Supratentorial primitive neuroectodermal tumors (SPNET) is otherwise a rare tumor and has been reported in 5 patients with *PMS2* mutations.
- Medulloblastoma reported in several families
  - Medulloblastoma is also associated with heterozygous APC mutations.
- Cells that are MMR-deficient are resistant to temozolomide.
  - MMR-D diagnosis may impact treatment decisions.

Wimmer & Etzler (2008)

Genetic Heterogeneity

- Pie chart based on families (not patients) reported in Wimmer & Etzler (2008).
- *PMS2* may be artificially high given study from UK evaluating consanguineous families (Vos et al, 2006).
- Early deaths in *MSH2/MLH1* families may have precluded molecular analysis.
  - *Some question lack of viability of some homozygous alleles in these genes.*



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Genotype/Phenotype Correlations

- There are clear genotype/phenotype correlations based on both:
  - Mutations in different genes (genetic heterogeneity)
  - Type of mutation in the genes (Allelic heterogeneity)
    - See delayed onset of tumors in patients carrying missense alleles
    - Type of tumors more similar to HNPCC

How many cases are we missing?

- Need to make pediatric oncology and neurosurgery clinicians aware of this diagnosis.
  - Atypical CALS are often missed by colleagues unless there is a targeted skin exam.
  - Pakistani families predominate due to:
    - High level of consanguinity
    - Founder *PMS2* mutations, e.g. R802X, in subset of families

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Microsatellite Instability in MMR-D

- Typical MSI studies compare "normal DNA" with tumor DNA.
  - MSI-high has been reported from "normal DNA" in MMR-D if small pool PCR techniques are used.
  - "normal" DNA appears MSI-stable in MMR-D if you use standard procedures.
- HNPCC-associated tumors show MSI-high
- The few brain tumors studied are MSI-stable

Comparing heterozygous versus biallelic mutations

- HNPCC
- Young-late adult tumors
- Hematopoietic tumors rare
- CRC most common
  - MIS+ seen in almost all tumors
- MSH2/MLH1>> PMS2/MSH6

- MMR-D
- Childhood onset tumors
- Hematopoietic tumors common
- Brain tumors
  - No evidence MIS+
- CRC less common
- Severity similar for all 4 genes

Rate limiting step for tumor formation?

- The likelihood that a second hit occurs in an MMR gene may be tissue specific:
  - Second hits appear rare in hematopoietic tissues unless pretreated.
  - Need for second hit makes childhood onset of CRC unlikely in adolescence. Diagnosis of CRC begins in twenties and continues throughout adulthood in HNPCC.
- The likelihood that a second hit occurs may vary among the MMR genes
  - Given cancer risk, we presume that *MLH1* and *MSH2* undergo 2nd hits more frequently then *MSH6* and *PMS2* thus resulting in a higher cancer risk in heterozygous cases for the former genes.

Questions?

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