Clinical Chemistry
Trainee Council

PEARLS OF
LABORATORY MEDICINE

Hereditary Colorectal Cancers

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Outline

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  - Lynch syndrome (Hereditary nonpolyposis colorectal cancer, HNPCC)
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  - Hamartomatous polyposis syndromes
- Lynch syndrome (HNPCC)
  - Criteria for genetic testing
  - Genetic basis
  - Molecular testing
- FAP
  - Genetic basis
  - MUTYH-associated polyposis
  - Molecular testing
- Hamartomatous polyposis syndromes
- Testing methods

Epidemiology

- Colorectal cancer is the 3rd most common cancer in both genders
- Hereditary colorectal cancer
  - Accounts for 5% of all colorectal cancers
  - Several generations of the family have colorectal cancer
  - Significantly increased risk for colorectal cancer and extra-colonic cancers (endometrial, renal pelvis, ureter, ovary, stomach, small intestine etc.)
  - Lynch Syndrome: 3%
  - FAP: 1%
  - Autosomal dominant genetic conditions

Lynch Syndrome

- Characterized by Henry T. Lynch, Professor of Medicine, Creighton University Medical Center in 1966
- Autosomal dominant
- Mean age of onset is 45 yrs, compared to 65 yrs for sporadic cancers
- Fewer polyps than FAP, but more aggressive
- 80% life-time risk for colorectal cancer
- 40-60% patients present with endometrial cancer first
- Increased risks for other gastrointestinal and reproductive system cancers

Criteria for Genetic Testing for Lynch Syndrome

- Identify patients who should be tested for microsatellite instability and mismatch repair genes
- Amsterdam Criteria (1990)
  - Number and type of family members, generations affected
  - Age of onset
  - Limited sensitivity
- Amsterdam II (1999)
- Bethesda (1997)
- Revised Bethesda (2004)
- Sensitivity increases, specificity decreases

Universal Testing Strategy

- Universal testing of all colorectal cancers for Lynch Syndrome
- Recommended by the EGAPP (Evaluation of Genomic Applications in Practice and Prevention) Working Group
- Adopted by many institutions
- Suggested to be more cost-effective than other approaches, but needs further studies

Genetic Basis for Lynch Syndrome

- DNA mismatch repair (MMR) gene defects
- Leads to microsatellite instability (MSI), accumulation of DNA sequence errors in multiple tandem repeats (microsatellites), affecting genes such as *APC*, *ras* and *p53*.
- MMR genes and related genes implicated (in order of descending frequency):
  - *MSH2*
  - *MLH1 (*90% together with *MSH2)*
  - *MSH6* (7-10%)
  - *PMS2* (< 5%*)*
  - *EPCAM (*just upstream of *MSH2,* deletion of polyA silences *MSH2,* 1%*)*

Molecular Testing for Lynch Syndrome

- Stepwise testing strategy
  - Step 1: Immunohistochemistry (IHC) or MSI testing of the tumor tissue
  - Step 2: Molecular genetic testing of the tumor for methylation and somatic *BRAF* mutations
  - Step 3: Molecular genetic testing of the MMR genes

Immunohistochemistry Testing for Lynch Syndrome

- Tumor tissues
- MMR gene expression
- Advantages
  - High sensitivity (92%)
  - Readily available
  - Identifies which MMR gene has mutations
  - Help reduce molecular genetic testing costs
- Limitations
  - Technical issues may result in equivocal staining patterns
  - May not identify tumors caused by some missense mutations
  - Less reliable on small tissue samples

Microsatellite Instability Testing for Lynch Syndrome

- Comparison of normal *vs* tumor tissues
  - positive in virtually all Lynch Syndrome cases
  - Positive in 10-15% of sporadic colon cancers
  - Differentiation requires tumor methylation and somatic *BRAF* mutation testing
  - A panel of 5 or more microsatellites
  - Gain/loss of repeats: unstable
  - $\geq$ 2/5 or >30% unstable markers: MSI-high
  - 1/5 or <30% unstable marker: MSI-low
  - 0/5 unstable marker: MSI-stable

Microsatellite Instability Testing for Lynch Syndrome

- Advantages
  - High sensitivity (93%, may be positive in IHC false negative cases)
  - Highly reproducible
  - Require very little tissue
- Limitations
  - Technical challenges in extremely mucinous tumors and tumors caused by *MSH6* mutations
  - Does not identify which MMR gene has mutations
  - Does not help reduce molecular genetic testing costs

MLH1 Promoter Hypermethylation

- Methylation of CpG sites in promoter silences transcription
- Microsatellite unstable
- Acquired epigenetic change, not heritable
- *MLH1* promoter hypermethylation may co-exist with germline *MLH1* mutations
- Positive results help eliminate, but do not completely rule out Lynch syndrome diagnosis

BRAF Mutation Testing for Lynch Syndrome

- *BRAF* c.1799T>A (p.V600E) mutation testing
- *BRAF* is part of EGFR signaling cascade
- Associated with decreased response to anti-*EGFR* therapies (cetuximab and panitumumab)
- *BRAF* mutation is rare in Lynch syndrome
- 70% of sporadic colon cancers with *MLH1* promoter hypermethylation carry the *BRAF* mutation
- Help differentiate between Lynch syndrome and sporadic colon cancer in MSI-H and *MLH1* IHC negative cases
- May determine eligibility for anti-*EGFR* therapy

Familial adenomatous polyposis (FAP)

- Characterized by the presence of more than hundreds or even thousands of precancerous polyps, or growths in the large intestine
- 50% people with FAP develop polyps by age 15, 95% develop polyps by age 35
- If the colon is not surgically removed, there is a 100% chance that some of the polyps will develop into cancer, usually by age 40
- Prophylactic colectomy is generally needed; NSAIDs has some chemoprevention effect
- Numerous extracolonic manifestations
- Attenuated FAP: average number polyps =30, diagnosed at later age

Genetic Basis for FAP

- Mutations in adenomatous polyposis coli (*APC*) gene
- Autosomal dominant
- *APC* gene important in *Wnt* pathway
- Up to 90% of FAP and <30% of attenuated FAP have detectable *APC* mutations
- 50-80% of FAP have point mutations leading to protein truncation, 8-12% have large genome deletions or duplications
- 20% of FAP have an apparent *de novo APC* mutation, some of which are due to somatic mosaicism

MUTYH-Associated Polyposis (MAP)

- mutY homolog gene (*MYH*, or *MUTYH*)
- Base excision repair gene, DNA damage repair
- Autosomal recessive
- Germline inactivation of both copies of *MUTYH* lead to transversion errors (*G/C* to *T/A*) in *APC*
- Two most common mutations p.Y179C and p.G396D described in northern European populations
- 20% cases do not have these two mutations
- Similar to attenuated FAP, but usually <100 polyps

Molecular Testing for FAP

- APC mutation testing should first be performed on a diagnosed family member (proband)
- Sequencing and deletion/duplication detection
- If mutation is found, perform testing in other family members
- If no mutation is found, consider other conditions such as *MUTYH*-Associated Polyposis
- Mutation carriers or at-risk family members with uninformative genetic test results are offered annual sigmoidoscopy or colonoscopy starting from age 10-12
- Recommendations for extracolonic surveillance

Hamartomatous Polyposis Syndromes

- Multiple hamartomatous polyps develop at a young age
- Predisposition to intestinal malignancies
- Subtypes:
  - Peutz-Jeghers (*STK11*)
  - Juvenile polyposis syndrome (*SMAD4, BMPR1A*)
  - PTEN hamartoma tumor syndromes (*PTEN*)
  - Hereditary mixed polyposis (*SCG5/GREM1*)
- Molecular testing is available at some clinical laboratories

Testing Methods

- Point mutations
  - Allele-specific PCR
  - Real-time PCR
  - PCR followed by sequencing
  - pyrosequencing
- Deletions/duplications
  - Southern blot
  - Multiplex ligation-dependent probe amplification (MLPA)
  - array comparative genomic hybridization (aCGH)
- Methylation
  - methylation-specific PCR
- Comprehensive gene panel
  - next-generation sequencing

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