COLON CANCER GENETICS

(FOR SURGEONS)

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DISCLAIMERS

- 1. I am a surgeon; of course I have nothing to disclose.
- 2. I am not a geneticist; which will be obvious after listening to this presentation.

KEY POINTS OF DISCUSSION

- Knudson's two hit hypothesis.
- Probability of developing colon cancer.
- Less common genetic syndromes.
- FAP.
- Lynch syndrome.
- Pedigrees.

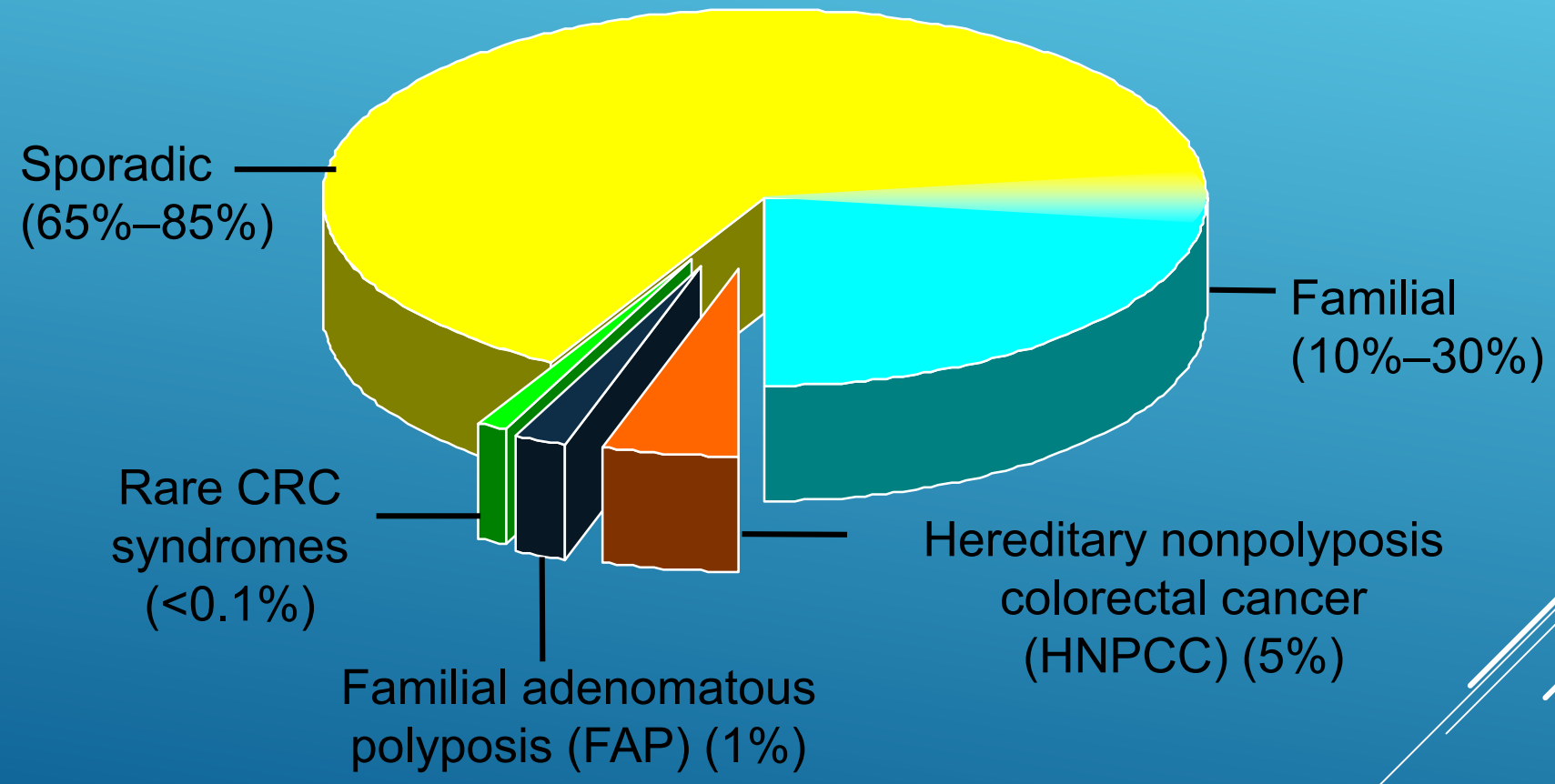
KNUTSON'S TWO HIT HYPOTHESIS

- Multiple "hits" in the DNA are required to cause cancer. The first hit is inherited, the second hit acquired.

MOLECULAR PATHWAYS LEADING TO COLON AND RECTAL CARCINOGENESIS

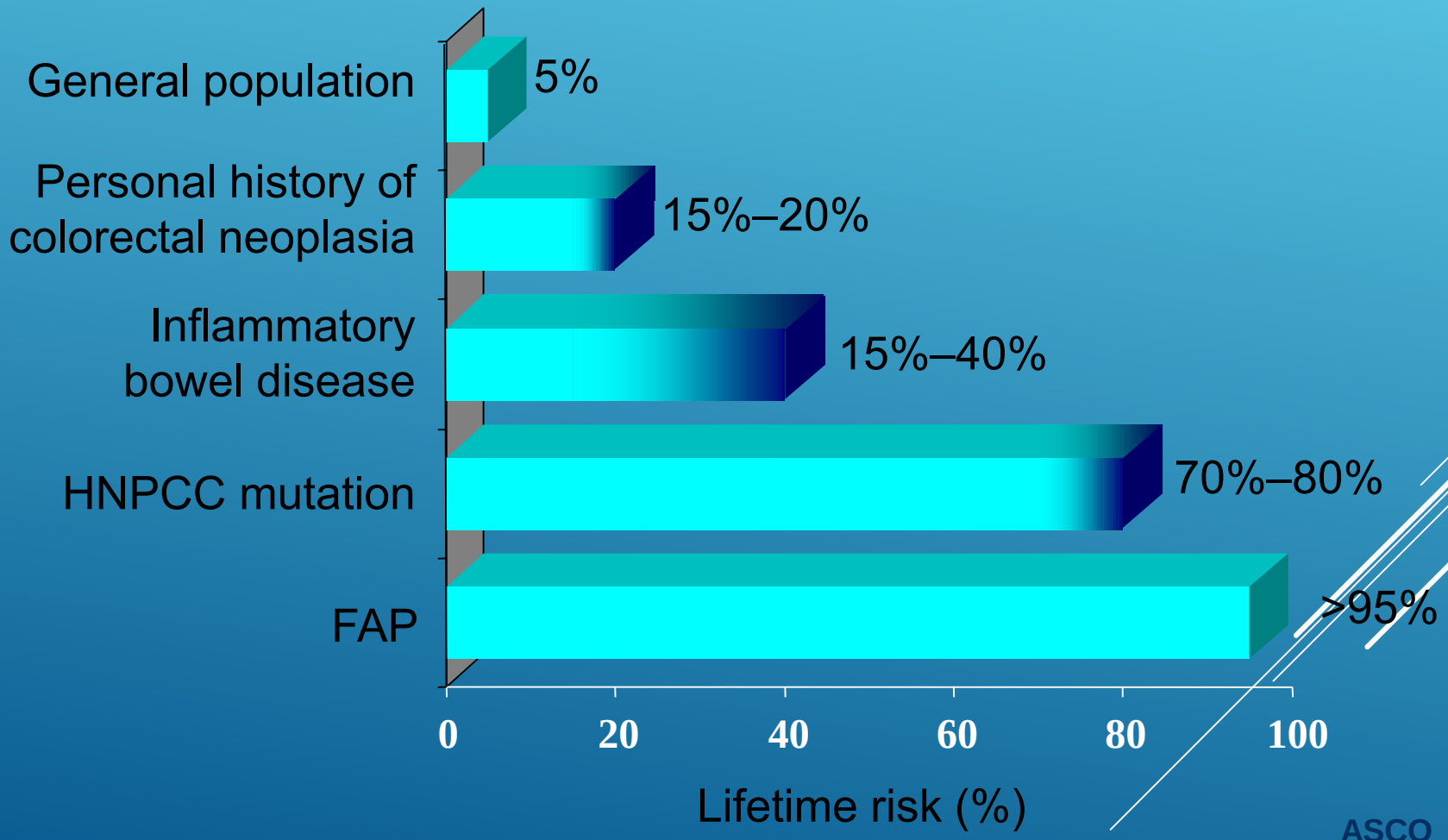
- Chromosomal instability (CIN), which accounts for approximately 85% of colorectal cancers.
- Microsatellite instability (MSI), or replication error, which accounts for approximately 15% of colorectal cancers.

SUSCEPTIBILITY to CRC



Adapted from Burt RW et al. *Prevention and Early Detection of CRC*, 1996

RISK OF COLORECTAL CANCER (CRC)



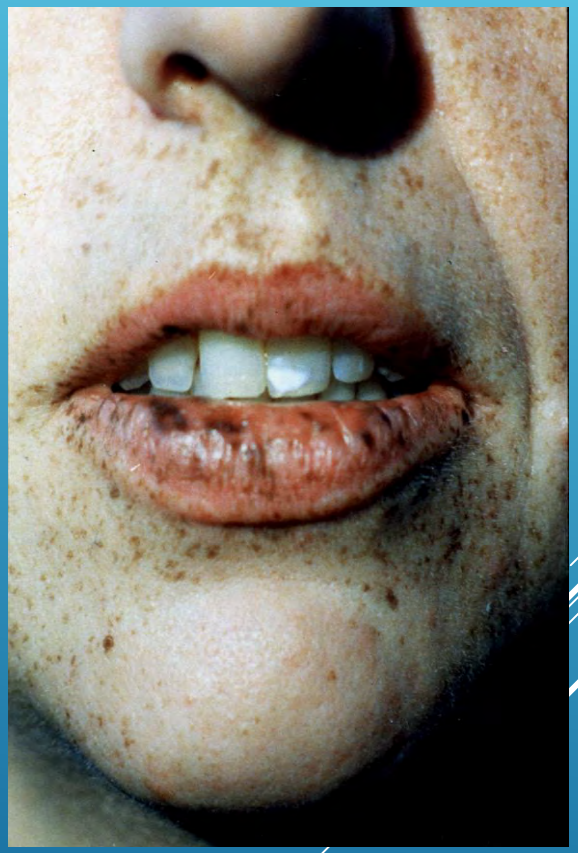
RELATIVE AND ABSOLUTE RISK OF CRC

Family History	Relative Risk	Absolute Risk, age 79
No family history	1	4%
1 first degree relative with adenoma	2.0	8%
1 first degree relative w CRC	2.3	9%
1 first degree relative with CRC before age 45	3.9	15%
> 1 first degree relative w CRC	4.3	16%

RARE GENETIC SYNDROMES

PEUTZ-JEGHERS SYNDROME

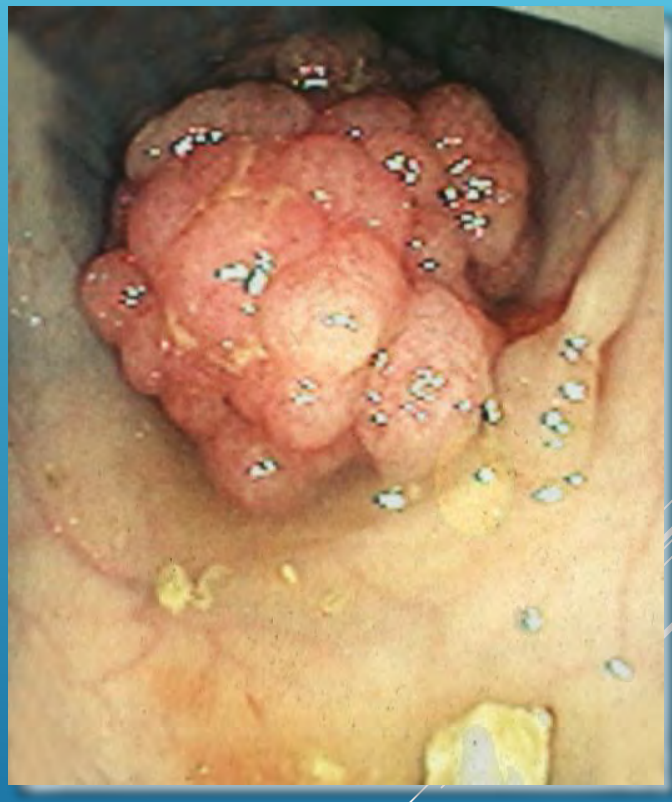
- *STK11* gene, chromosome 19
- GI hamartomas
- Characteristic pigmentation
- 2%–13% lifetime CRC risk
- Other cancers include small bowel, pancreas, ovary and other sex-cord tumors



Jenne DE, et al. Nature Genetics 18:38, 1998

JUVENILE POLYPOSIS COLI

- Autosomal dominant (rare)
- Linked to *PTEN* in some families; 18q in others
- Juvenile polyps with mixed adenomatous histology



Olschwang S et al. *Nature Genetics* 18:12, 1998

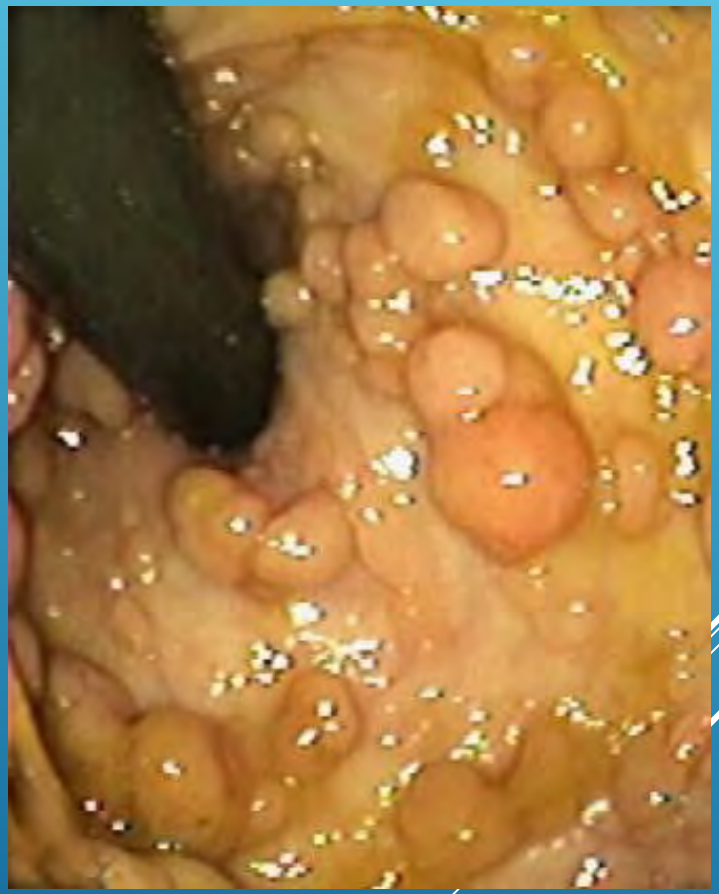
MYH POLYPOSIS (MAP)

- Caused by mutations in the *MyH* gene on the short arm of Chromosome 1.
- MUTYH glycosylase is involved in oxidative DNA damage repair.
- There are two common mutations are *Y165C* and *G382D*.
- Autosomal recessive.
- Risk of cancer at age 20 – 50.
- Screening colonoscopy starting age 18.
- Increased risk for stomach cancer.

FAMILIAL ADENOMATOUS POLYPOSIS (FAP)

CLINICAL FEATURES OF FAP

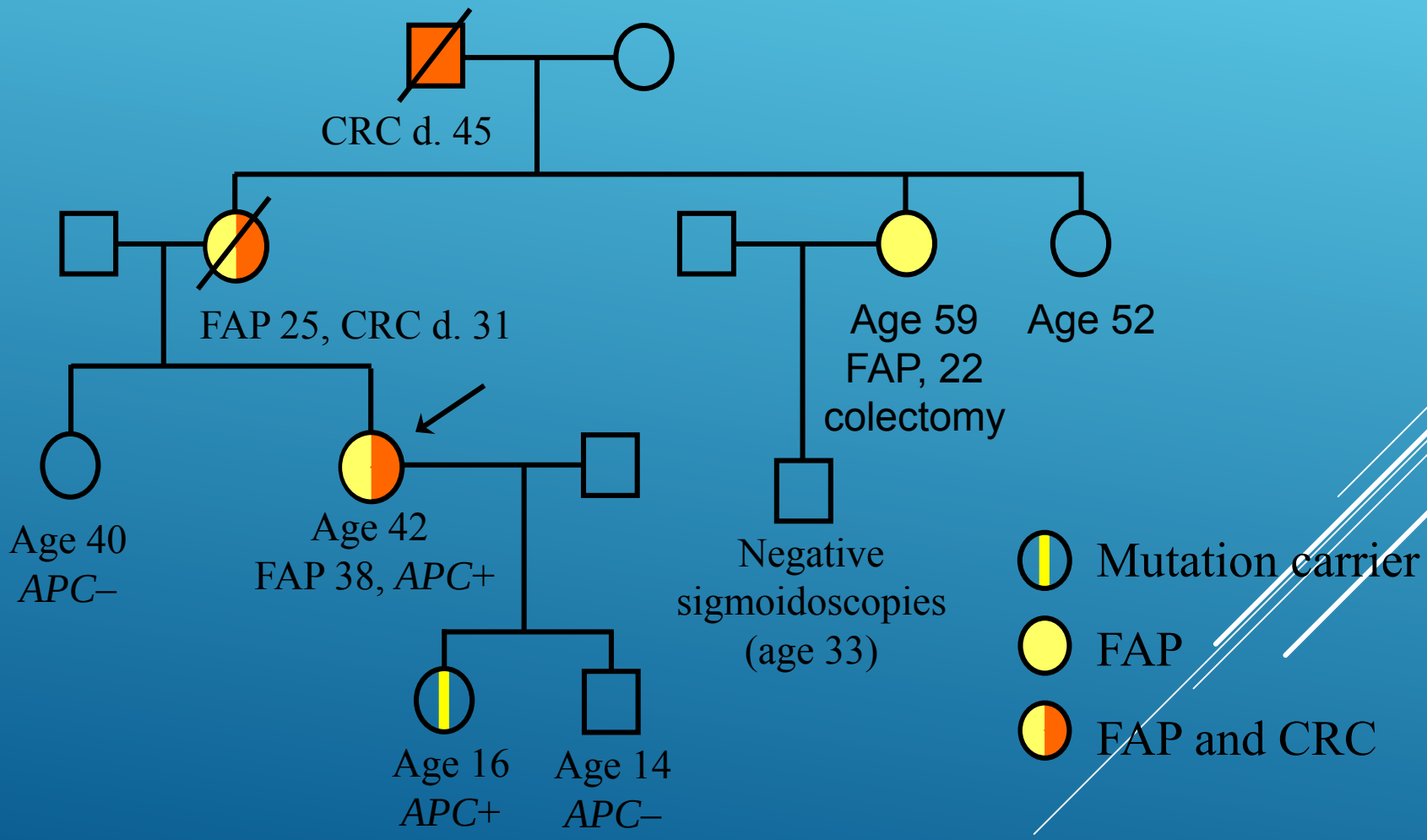
- Estimated penetrance for adenomas >90%
- Risk of extracolonic tumors (upper GI, desmoid, osteoma, thyroid, brain, other)
- Ophthalmolic lesions may be present (CHRPE)
- Untreated polyposis leads to 100% risk of cancer



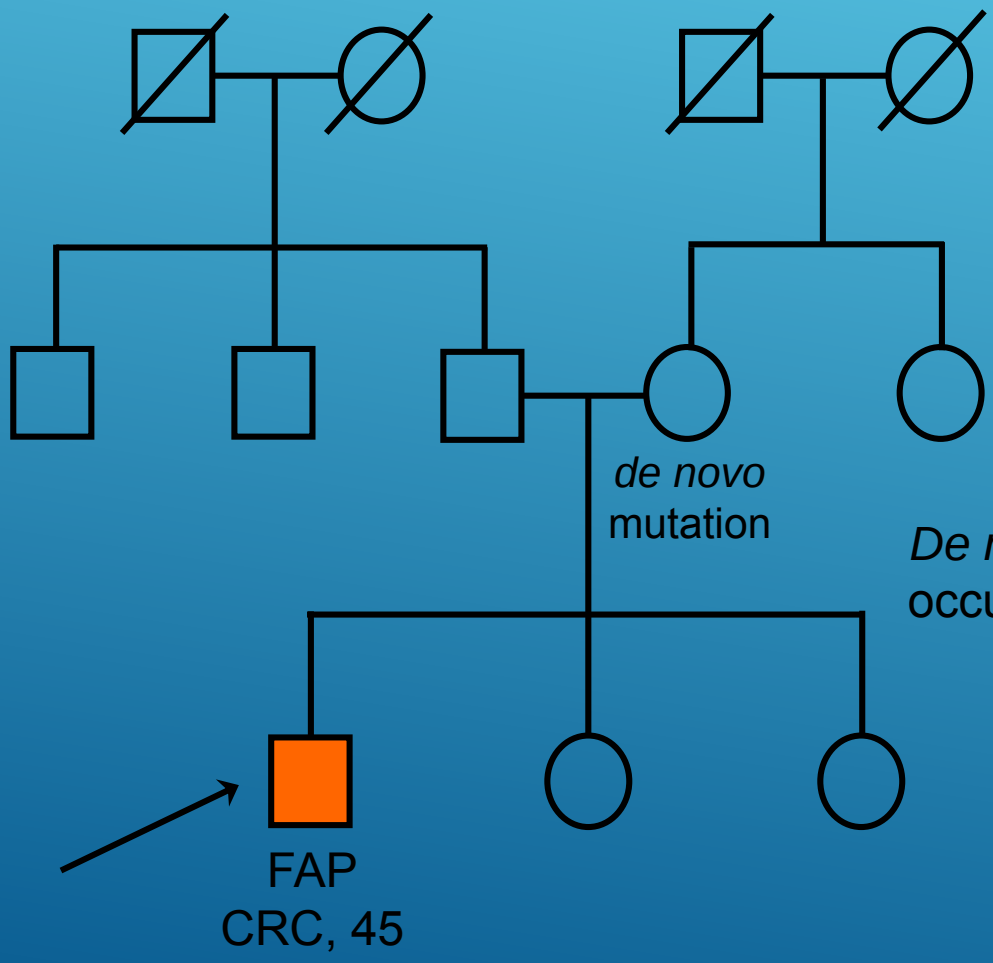
GENETICS OF FAP

- Autosomal dominant inheritance
- Caused by mutations in *APC* tumor suppressor gene on chromosome 5q
- Up to 30% of patients have *de novo* germline mutations
- Most families have unique mutations
- Most mutations are protein truncating

FAP FAMILY WITH *APC* MUTATION

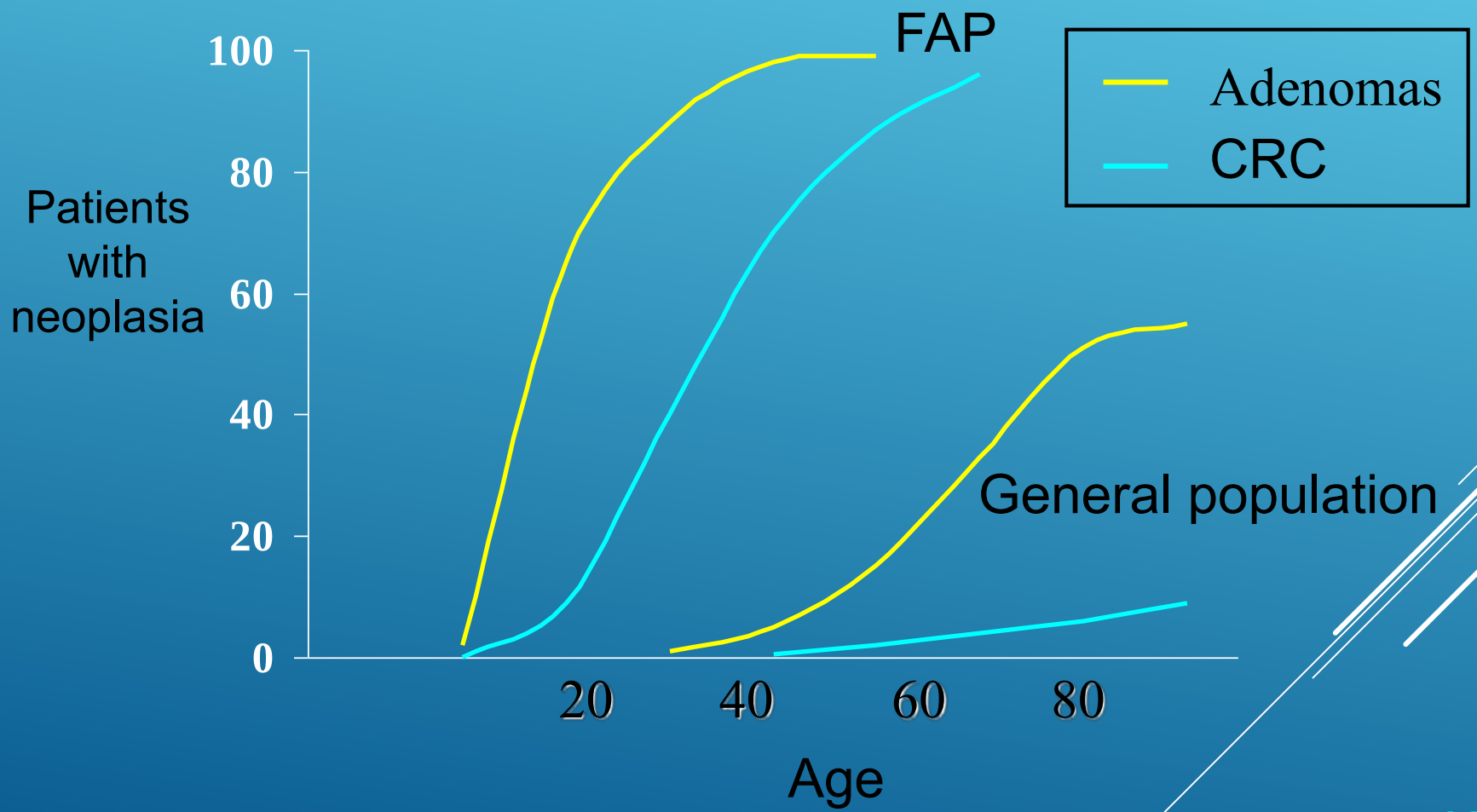


DE NOVO GERMLINE MUTATIONS IN FAP

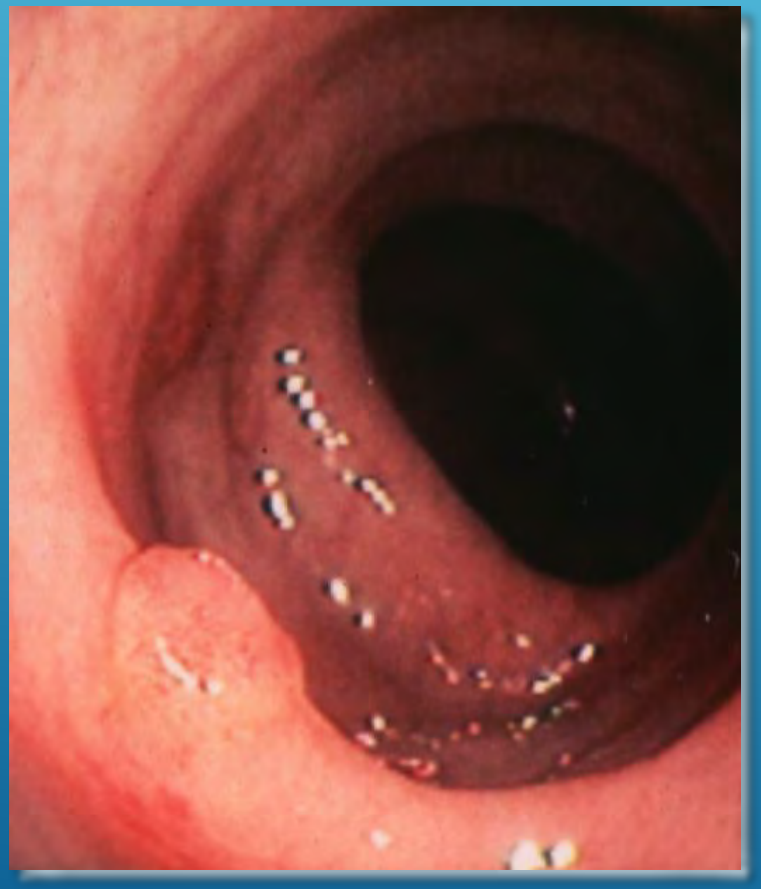


De novo germline mutations occur in ~30% of FAP cases

FAP: AGE AND DEVELOPMENT OF ADENOMAS AND CRC



ATTENUATED FAP



- Later onset (CRC ~age 50)
- Few colonic adenomas
- No retinal lesions
- UGI lesions
- Associated with mutations at 5' and 3' ends of *APC* gene

INDICATIONS FOR *APC* GENE TESTING

- Molecular diagnosis of FAP in patients who present with:
  - polyposis (>100 adenomas)
  - attenuated FAP
- Predictive testing for FAP in blood relatives of persons with FAP or known *APC* mutations

Giardiello FM et al. *N Engl J Med*, 336:823, 1997

GENETIC SUSCEPTIBILITY TESTING FOR FAP

Benefits	Limitations
▸ Identifies non-mutation carriers, who require only population screening for CRC	▸ False-negative results may lead to underestimated CRC risk
▸ Identifies *APC* mutation carriers, in whom early disease intervention may be lifesaving	▸ *APC* mutation tests are not informative in all FAP families

EARLY MANAGEMENT OF FAP

- Endoscopy beginning at age 10
- Removal of polyps
- Abdominal U/S for hepatoblastoma (birth to 5 yrs)
- Dilated eye exam (ophthalmologist)
- Upper EGD: when colon polyps develop or age 25

MANAGEMENT OF FAP

- Annual thyroid exam
- If symptoms develop:
  - Panorex of jaw and/or skull X-ray
  - Abdominal and pelvic CT
- Total colectomy is usually necessary when polyps become too numerous to remove with standard techniques often by age 25.

SURGICAL OPTIONS

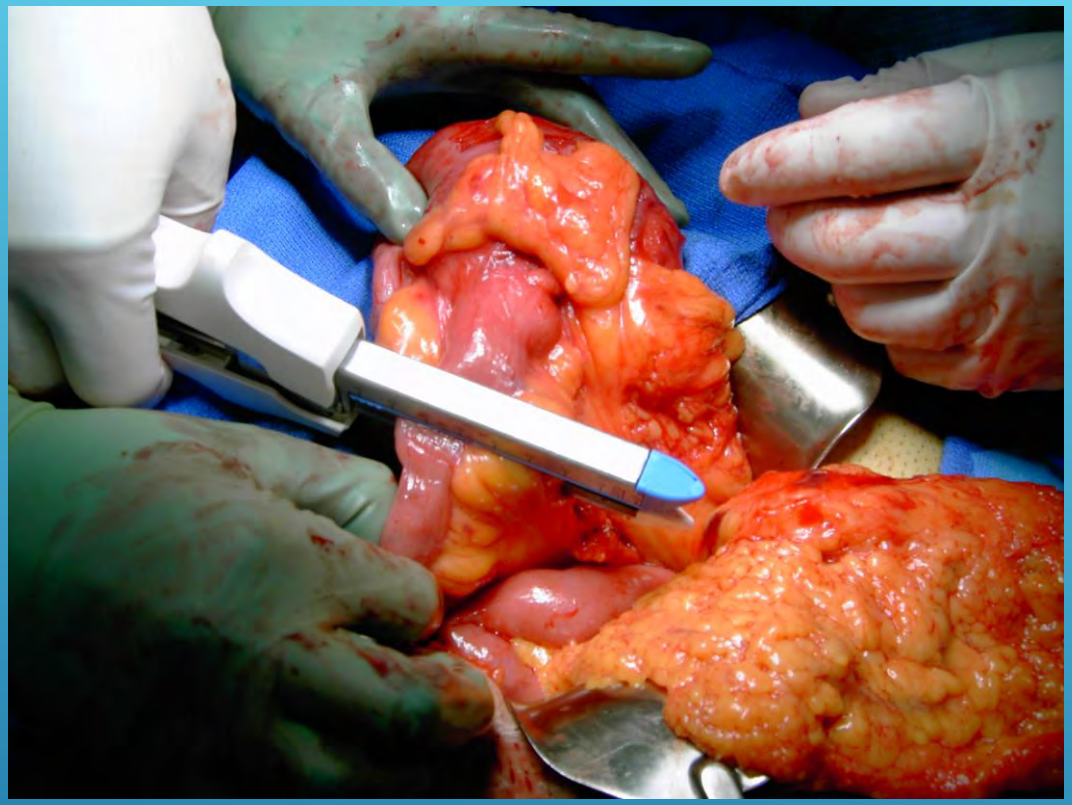
- Total colectomy with ileo-rectal anastomsis.
- Total proctocolectomy with end ileostomy.
- Total proctocolectomy with ileo-anal pouch reconstruction.

TECHNICAL CONSIDERATIONS ILEO-ANAL RECONSTRUCTION

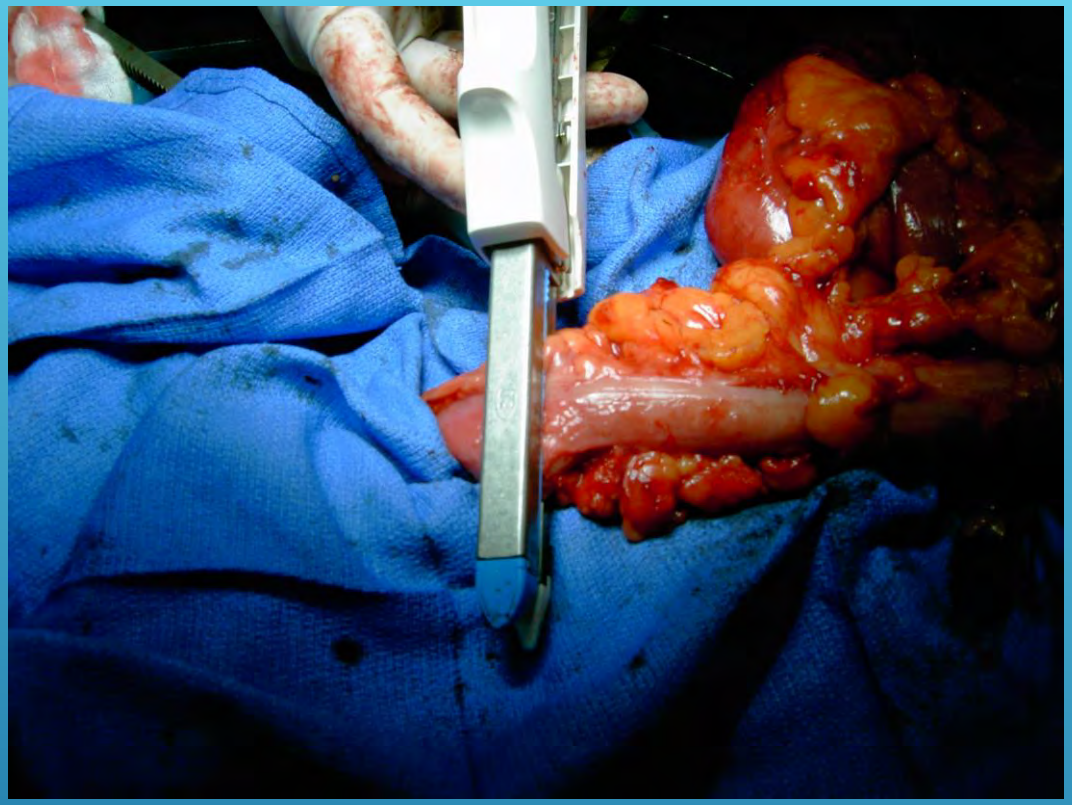
- S-pouch vs. J-pouch
- Mucosectomy vs. double staple
- Temporary ileostomy vs. no ileostomy

AN OPERATION IN 5 STEPS

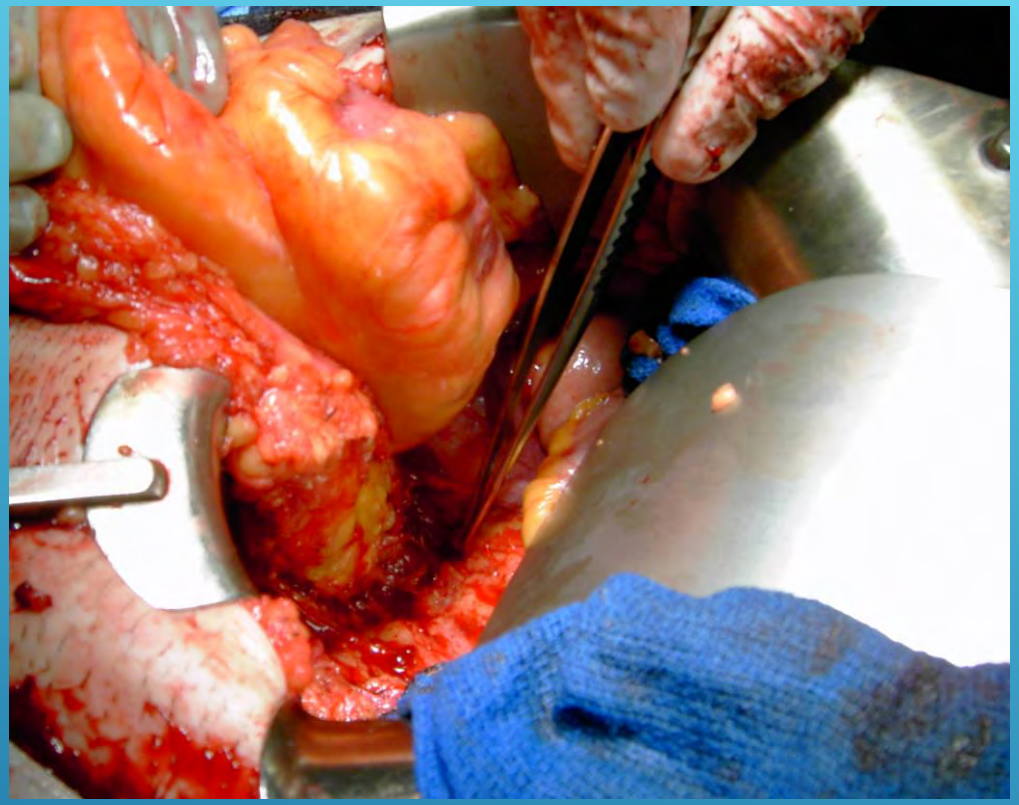
- Step 1: Colectomy
- Step 2: Proctectomy
- Step 3: Ileal Pouch
- Step 4: Anastomosis
- Step 5: Loop Ileostomy



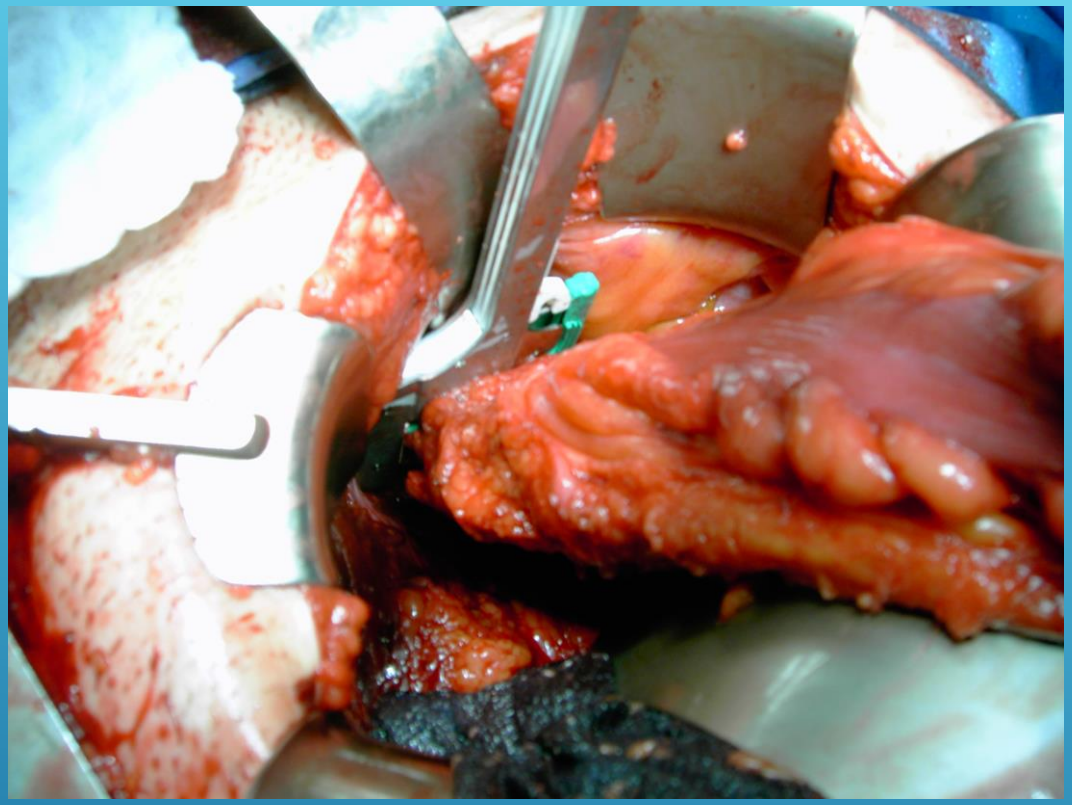
COLECTOMY: ILEAL DIVISION



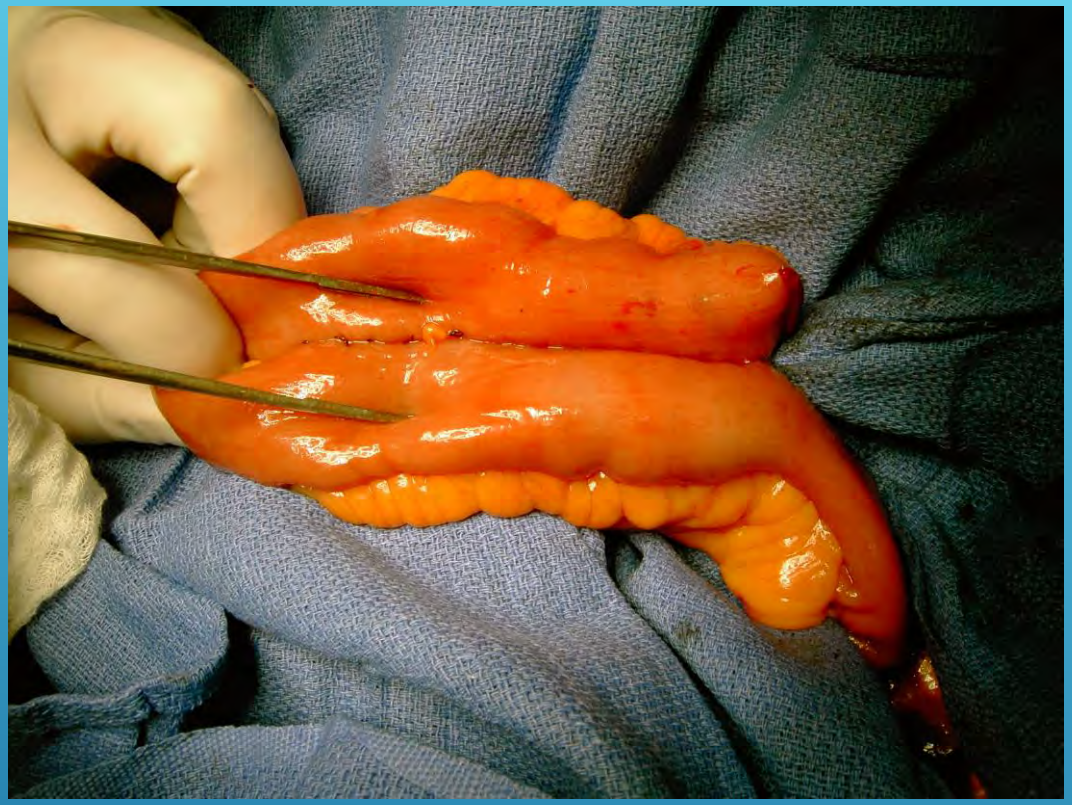
COLECTOMY: SIGMOID DIVISION



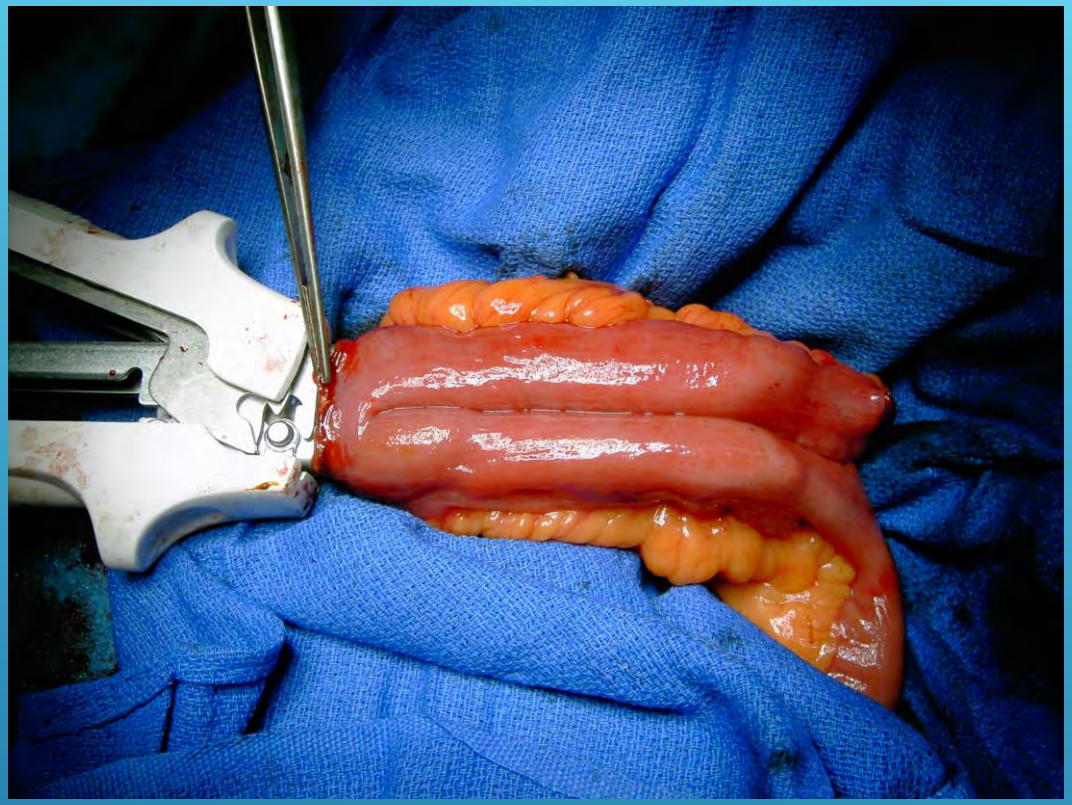
PROCTECTOMY: POSTERIOR DISSECTION



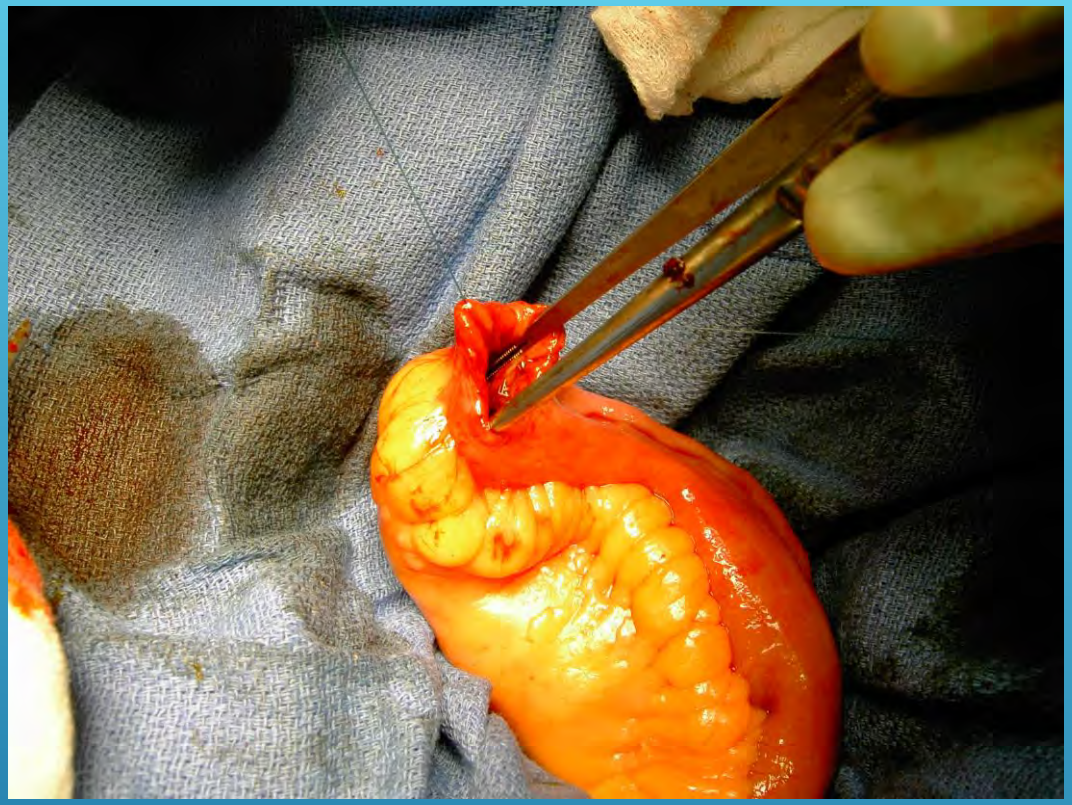
PROCTECTOMY: DISTAL DIVISION



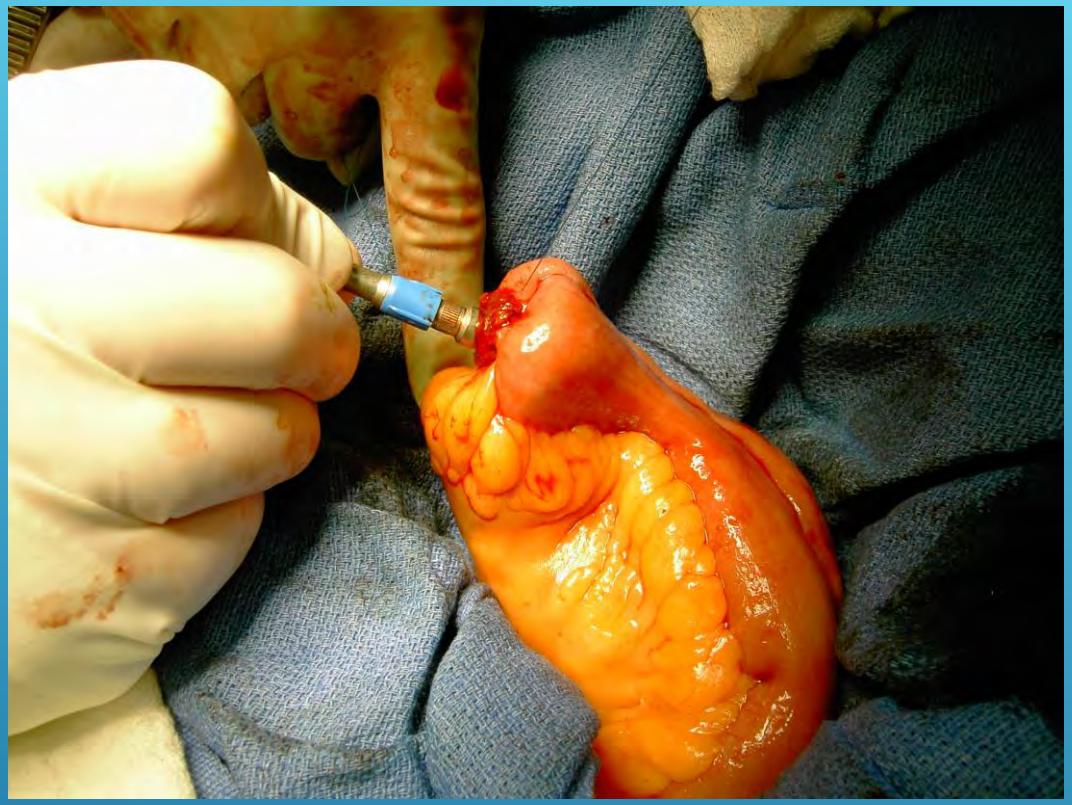
ILEAL POUCH: PLICATION



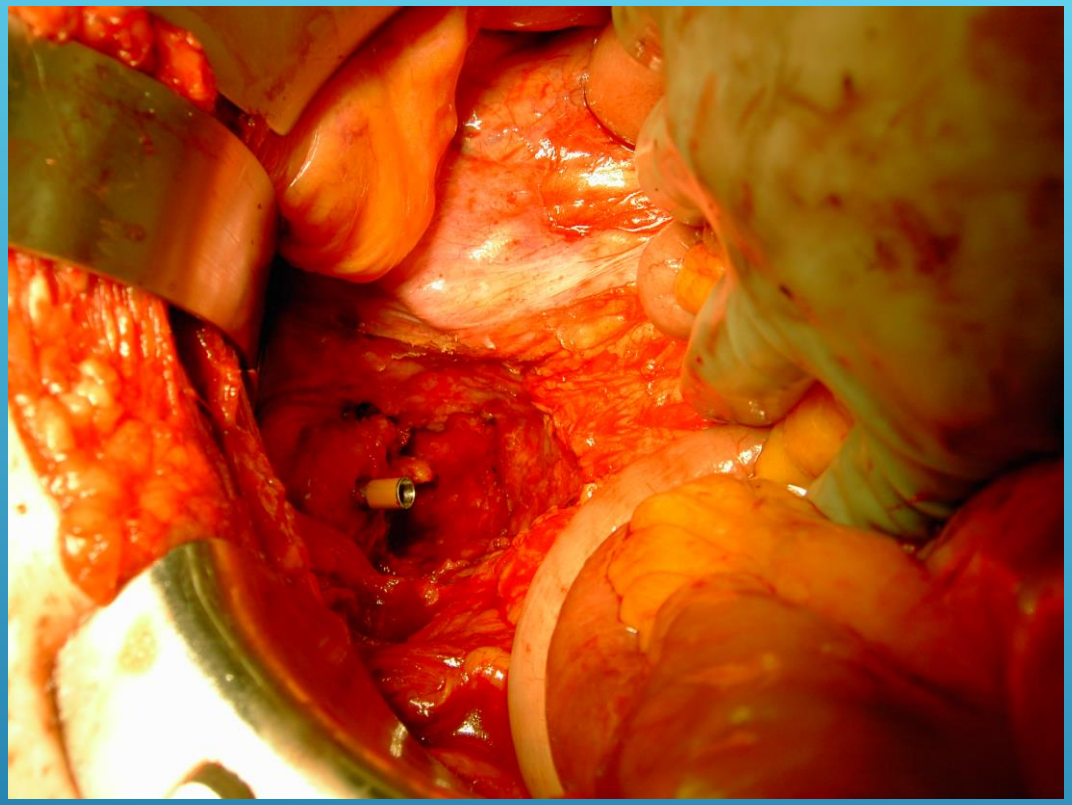
ILEAL POUCH: COMMON WALL DIVISION



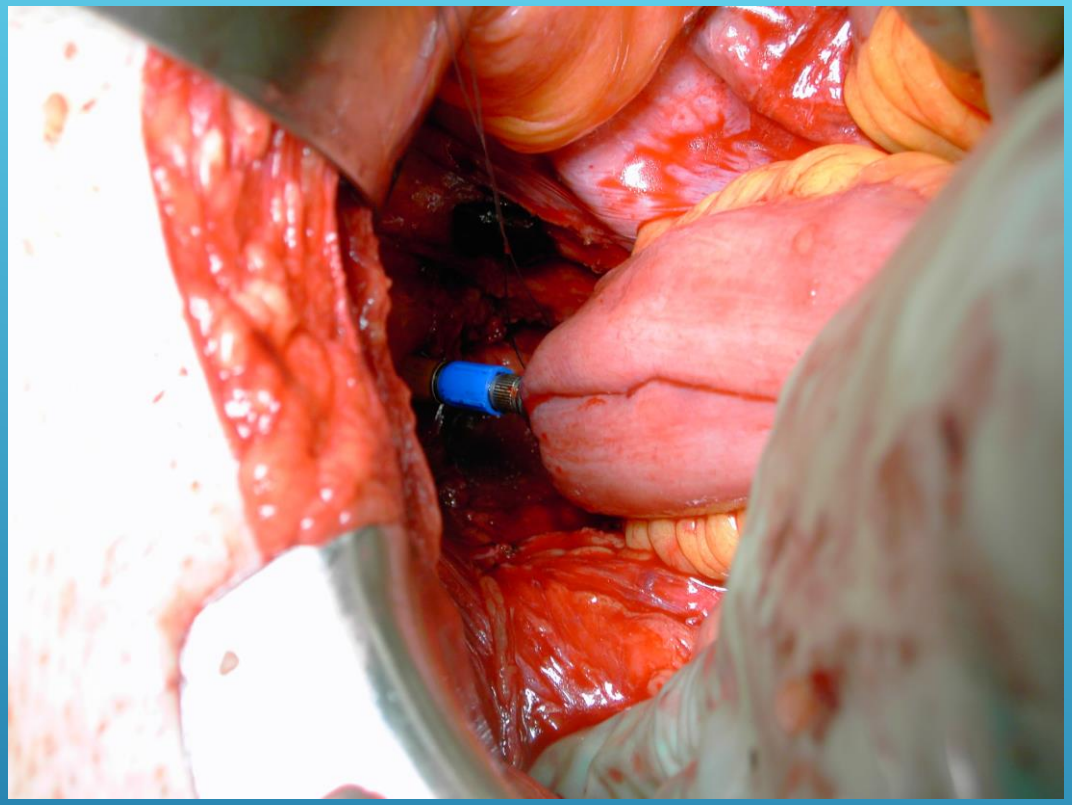
ILEAL POUCH: PLACING THE PURSTRING



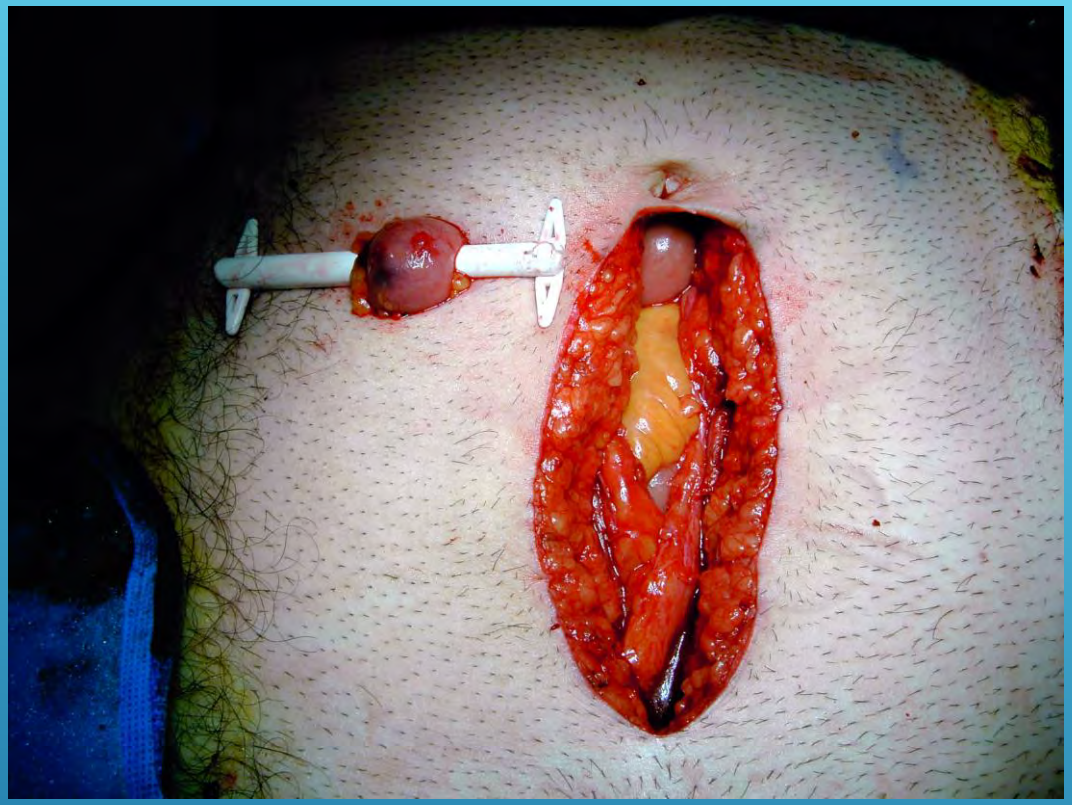
ILEAL POUCH: PLACING THE ANVIL



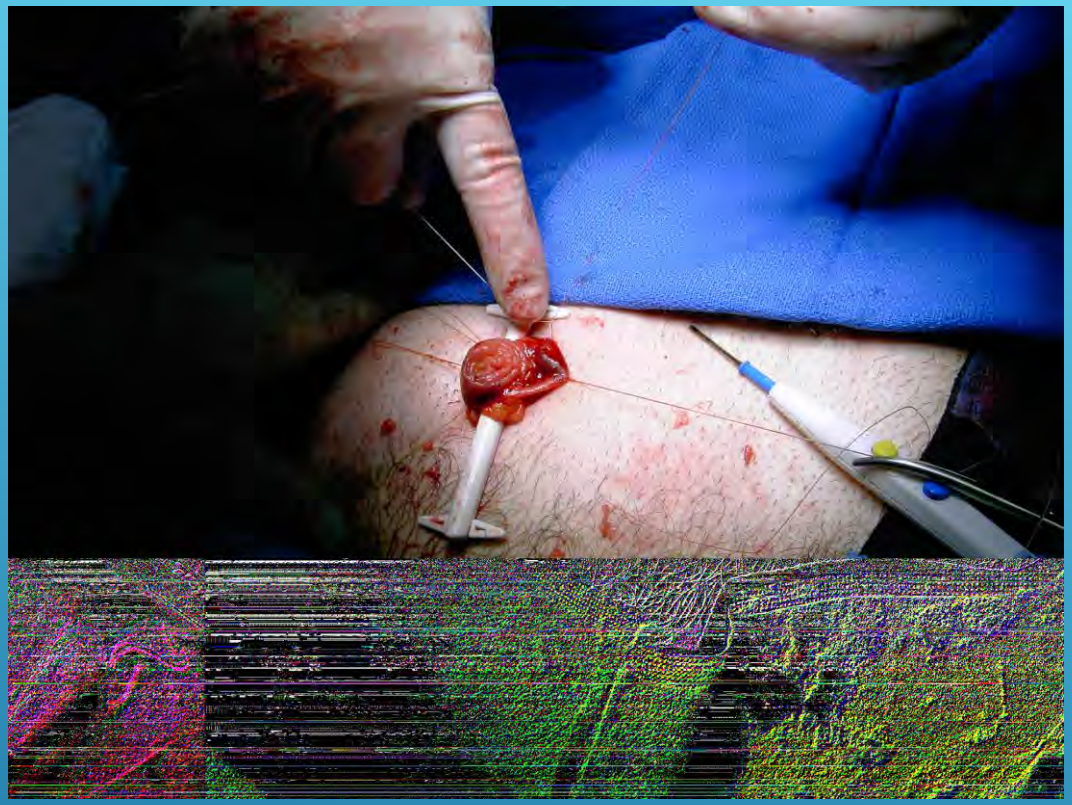
ANASTOMOSIS: DISTAL INSERTION



ANASTOMOSIS: ILEOPROCTOSTOMY

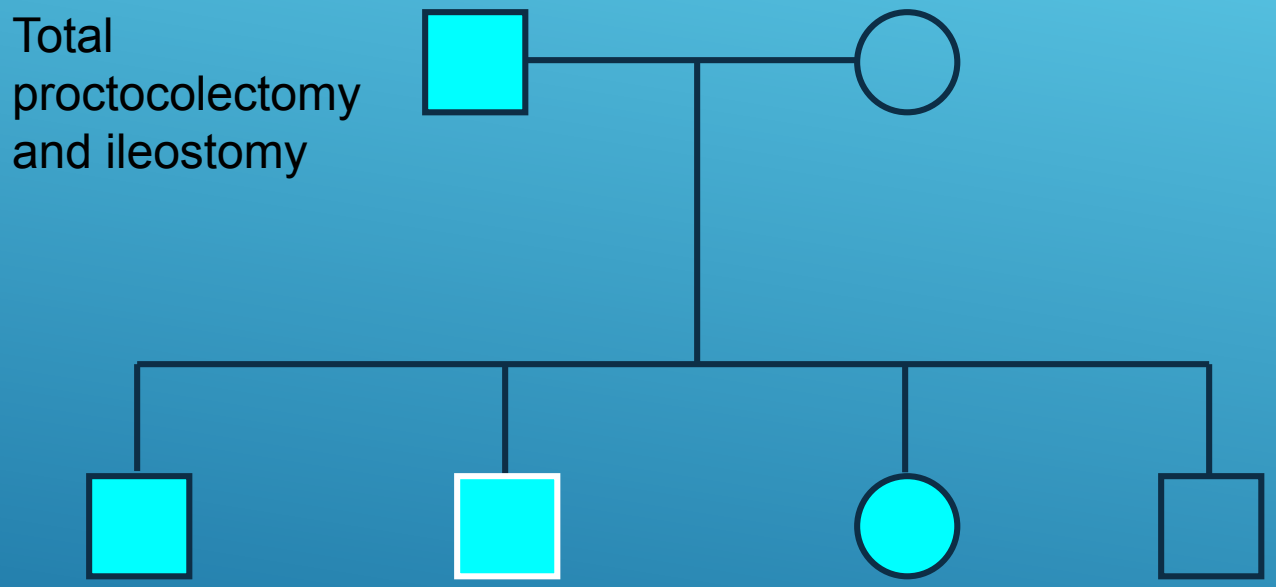


ILEOSTOMY: TEMPORARY STOMA PLACEMENT

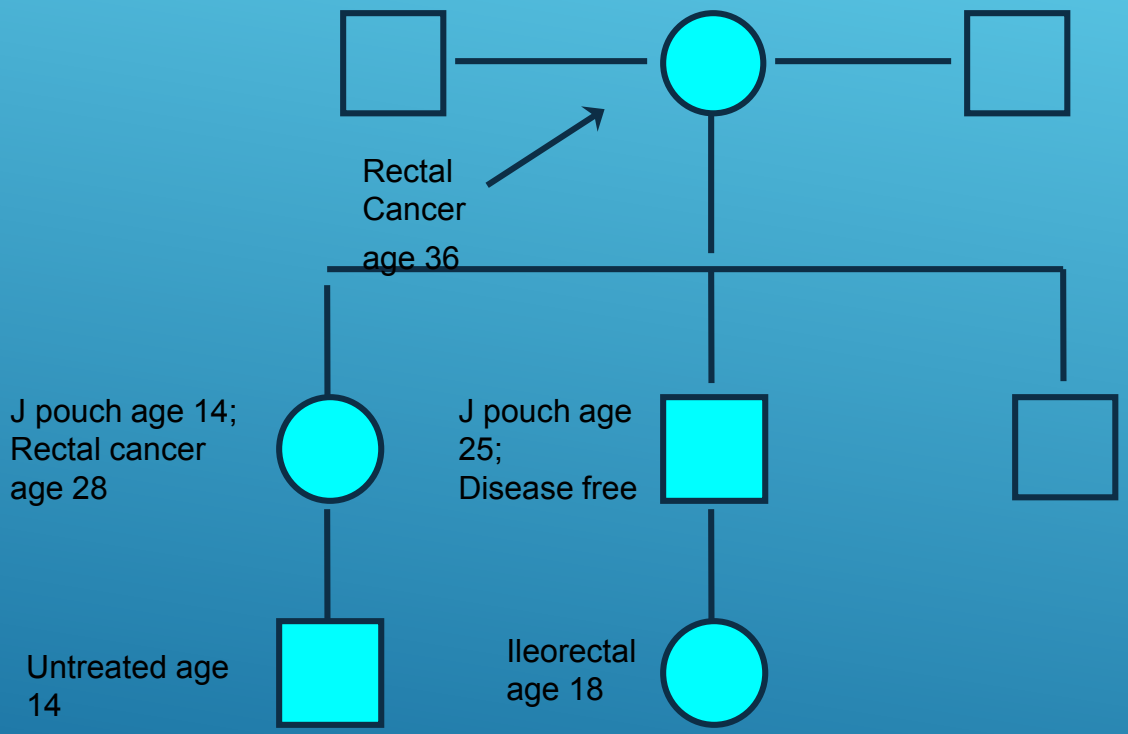


ILEOSTOMY: MATURING THE STOMA

FAP Pedigree - MC



FAP Pedigree - MM



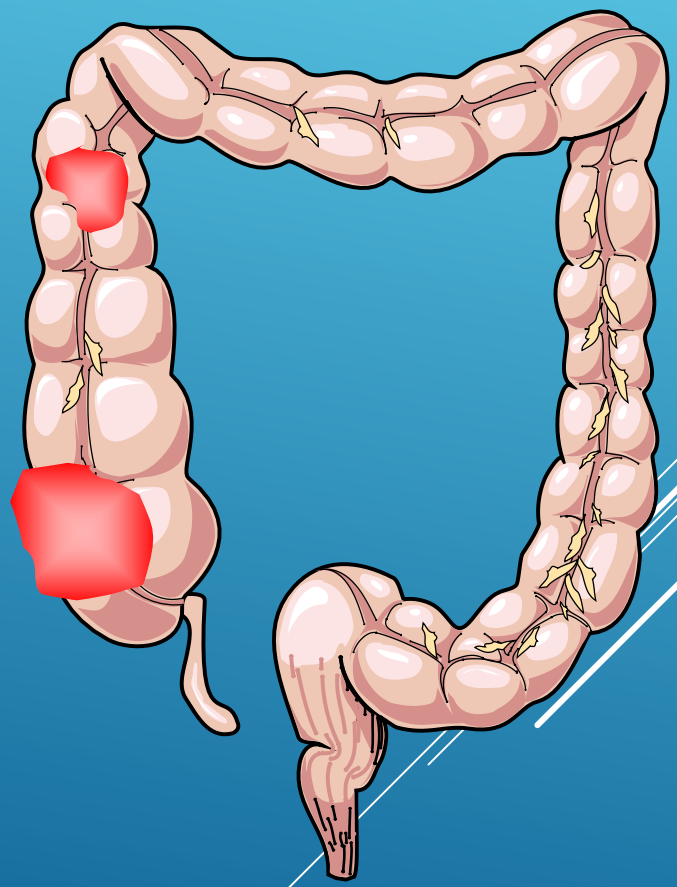
FAP IMPORTANT POINTS

- Wide range of expression.
- May be de novo mutation.
- Early diagnosis and treatment.
- Surgical approach should be tailored to the patient.
- Lifetime close follow-up is needed.
- High risk of developing malignancy.

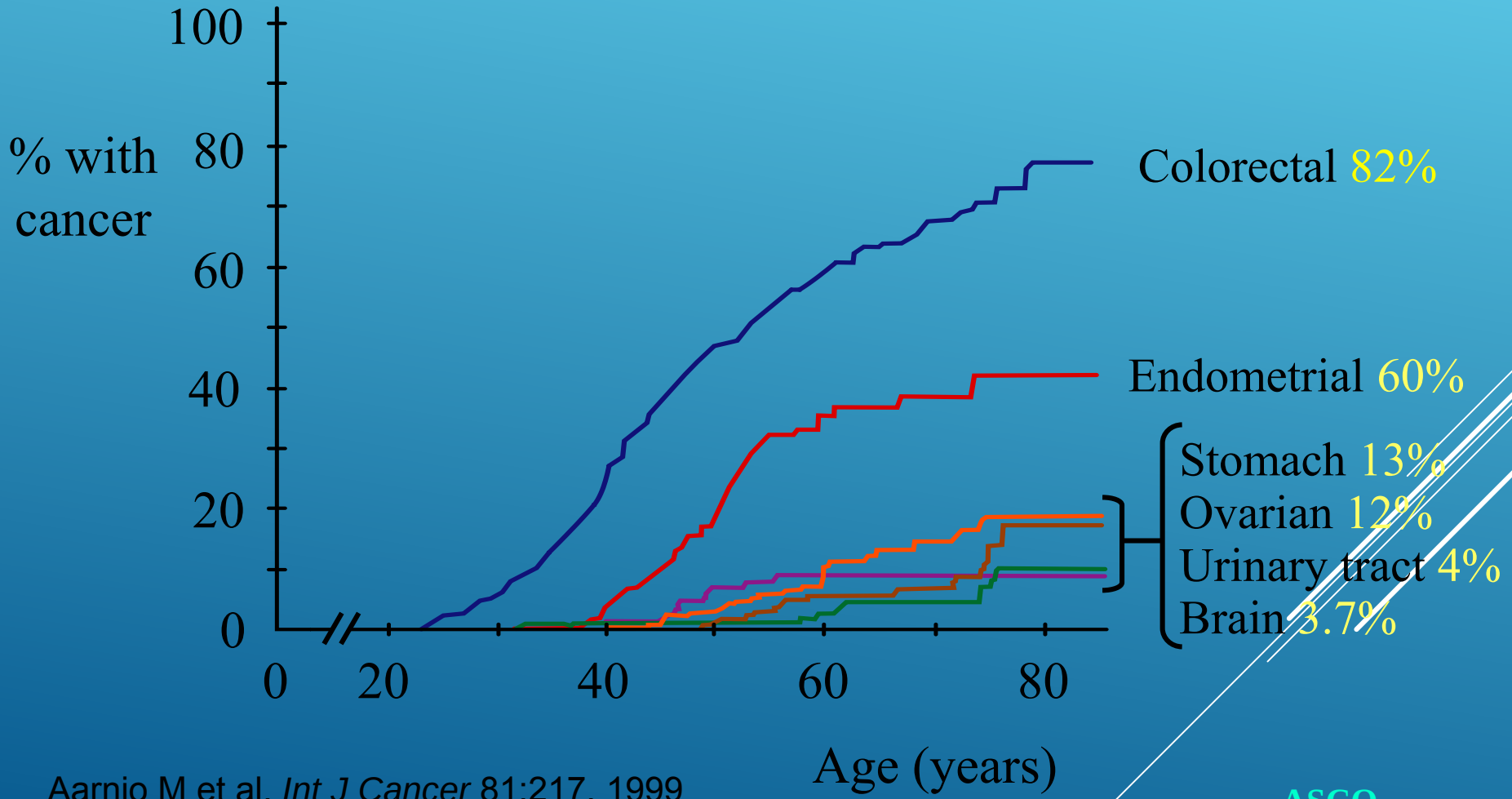
HEREDITARY NONPOLYPOSIS COLORECTAL CANCER

Clinical Features of HNPCC

- Early but variable age at CRC diagnosis (~45 years)
- Tumor site in proximal colon predominates
- Extra-colonic cancers: endometrium, ovary, stomach, urinary tract, small bowel, bile ducts, sebaceous skin tumors



Cancer Risks in HNPCC



Aarnio M et al. *Int J Cancer* 81:217, 1999

Amsterdam Criteria II

- 3 or more relatives with verified HNPCC-associated cancers* in family
- One case a first-degree relative of the other two
- Two or more generations
- One CRC by age 50
- FAP excluded

*HNPCC associated cancers:
CRC, endometrial, small bowel, ureter, renal pelvis

Vasen HFA et al. *Gastroenterology* 116:1453, 1999

BETHESDA GUIDELINES- REVISED 2004

To identify patients for MSI testing

Amsterdam criteria or

- Individual with CRC dx <50 yo
- Synchronous or metachronous CRC, or other HNPCC-associated tumors regardless of age
- CRC with MSI-H histology dx <60 yo
- CRC with ≥1 FDR with an HNPCC-associated tumor, with one cancer dx <50
- CRC with ≥2 FDRs or SDRs with an HNPCC-associated tumor, regardless of age

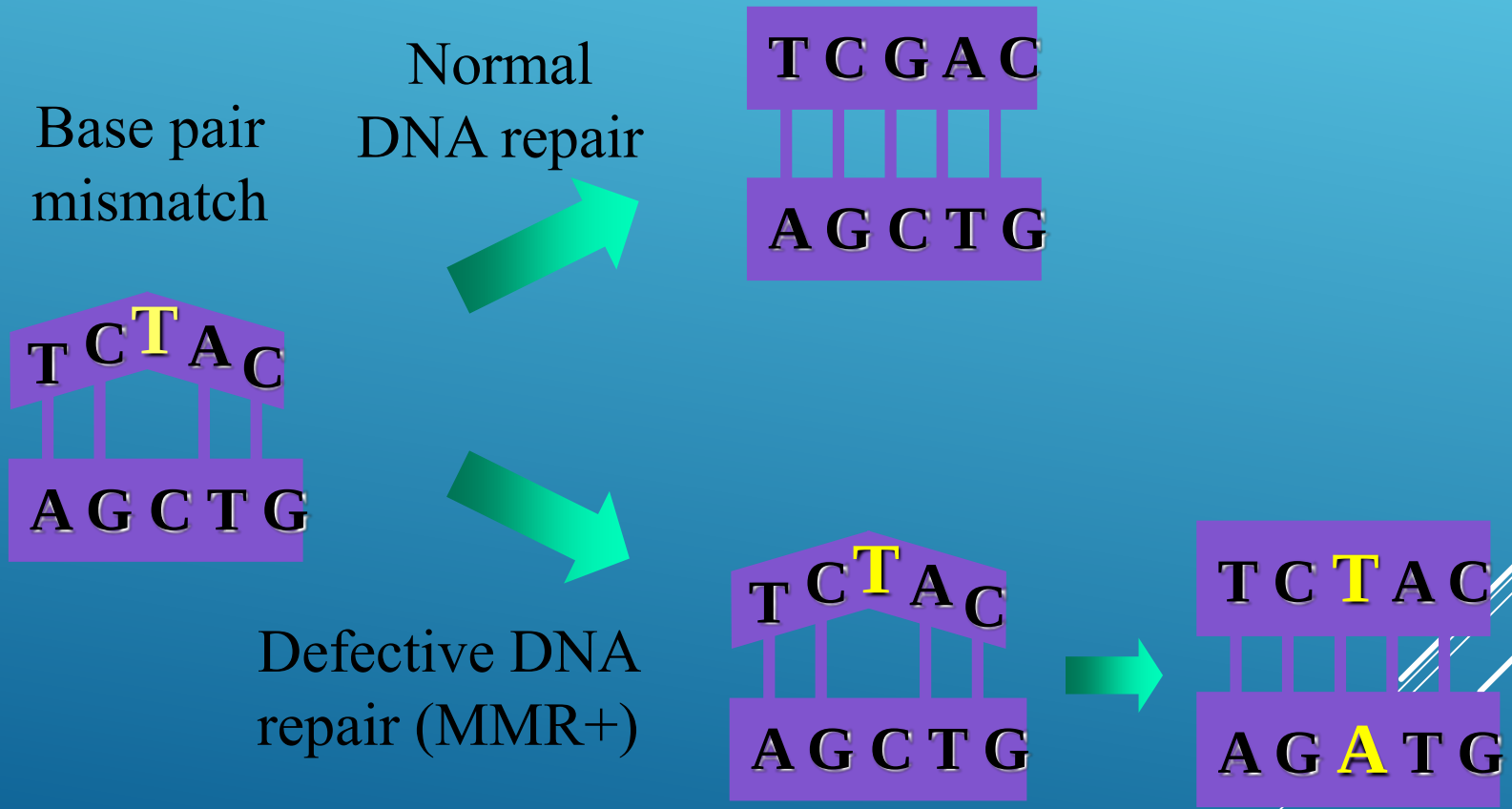
International Workshop HNPCC
Dec 2002, Bethesda, MD

Umar A, et al. JNCI. 2004;96(4):261-268.

GENETIC FEATURES OF HNPCC

- Autosomal dominant inheritance
- Penetrance ~80%
- Genes belong to DNA mismatch repair (MMR) family
- Genetic heterogeneity (*MLH1, MSH2, MSH6, PMS1, PMS2*)
- Mutations in MMR genes lead to microsatellite instability
- MMR proteins are missing in the tumor tissue due to two-hit hypothesis making immunohistochemical staining useful

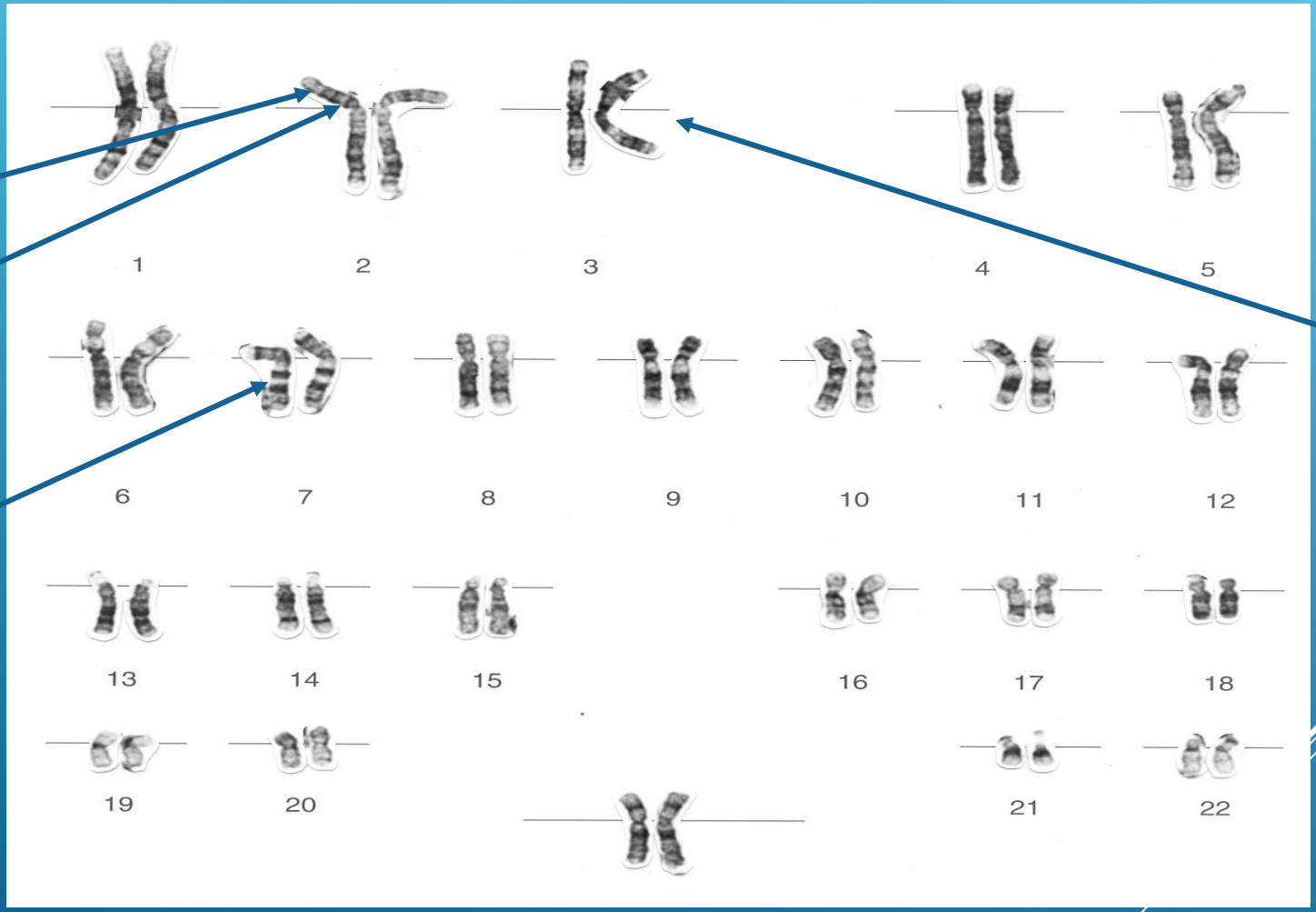
MSI IS CAUSED BY FAILURE OF MISMATCH REPAIR (MMR) GENES



MICROSATELLITE INSTABILITY (MSI)

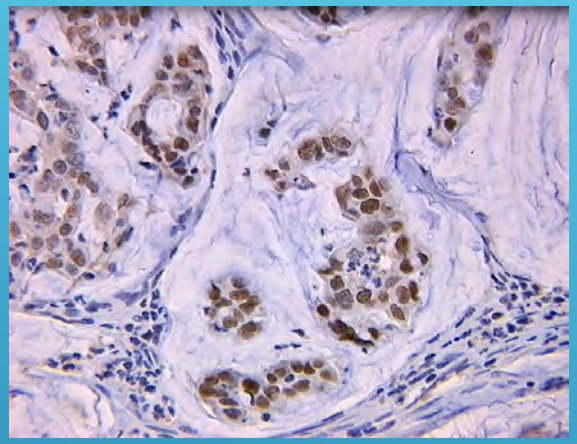
- 95% of HNPCC tumors are MSI+
- 10%–15% of sporadic CRCs are MSI+
- 2-3% of CRCs are due to HNPCC
- Therefore, ~1 in 5 MSI+ CRC is due to HNPCC
- Others due to acquired methylation of MLH1 promoter

HEREDITARY NONPOLYPOSIS COLORECTAL CANCER (HNPCC)

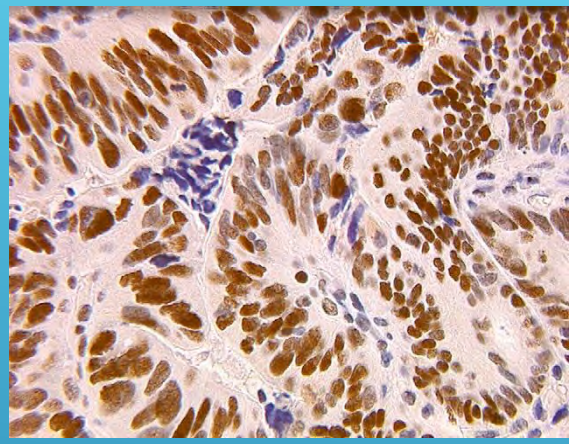


IMMUNOHISTOCHEMISTRY

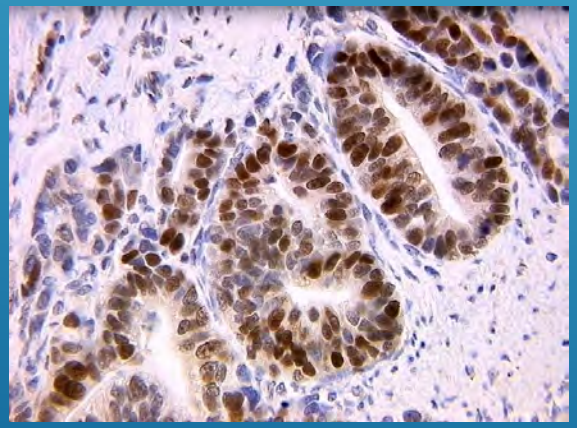
- Identify MMR proteins normally present
- If protein is absent, the gene is not being expressed (mutation or methylation)
- Helps direct gene testing by predicting likely involved gene
- If abnormal IHC, the patient is MSI+



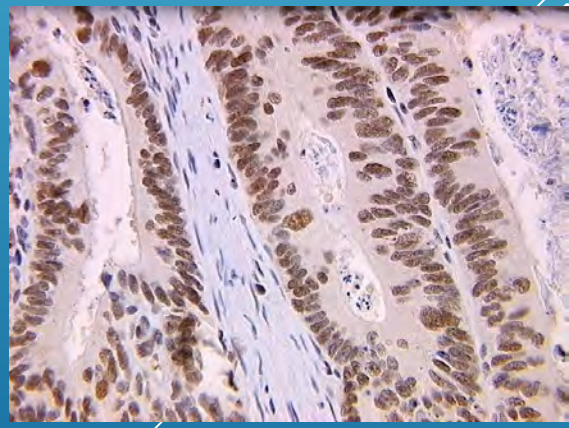
MLH1



MSH2

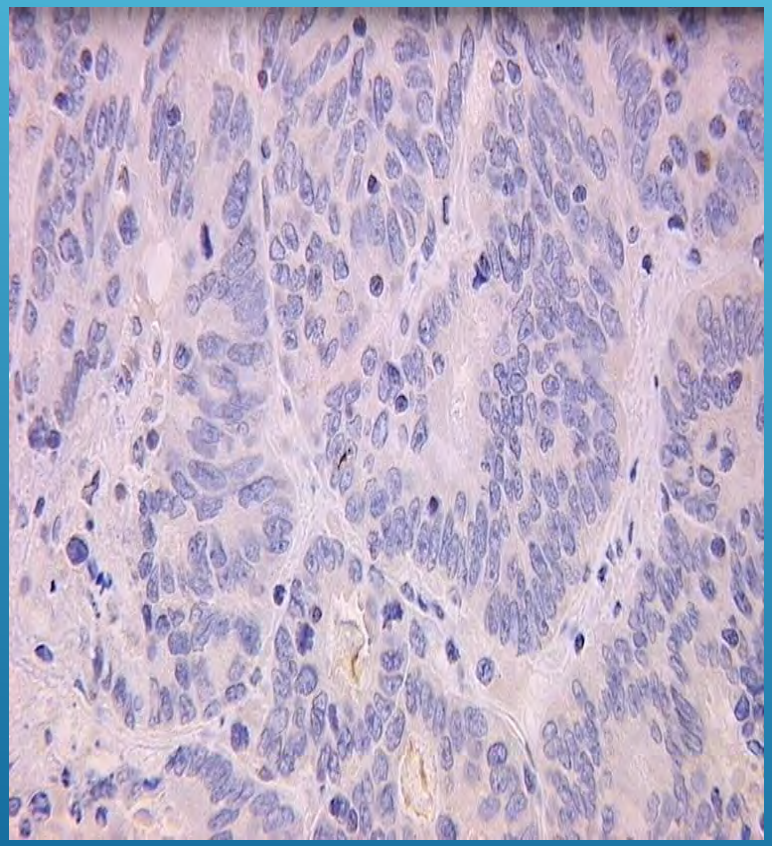


MSH6

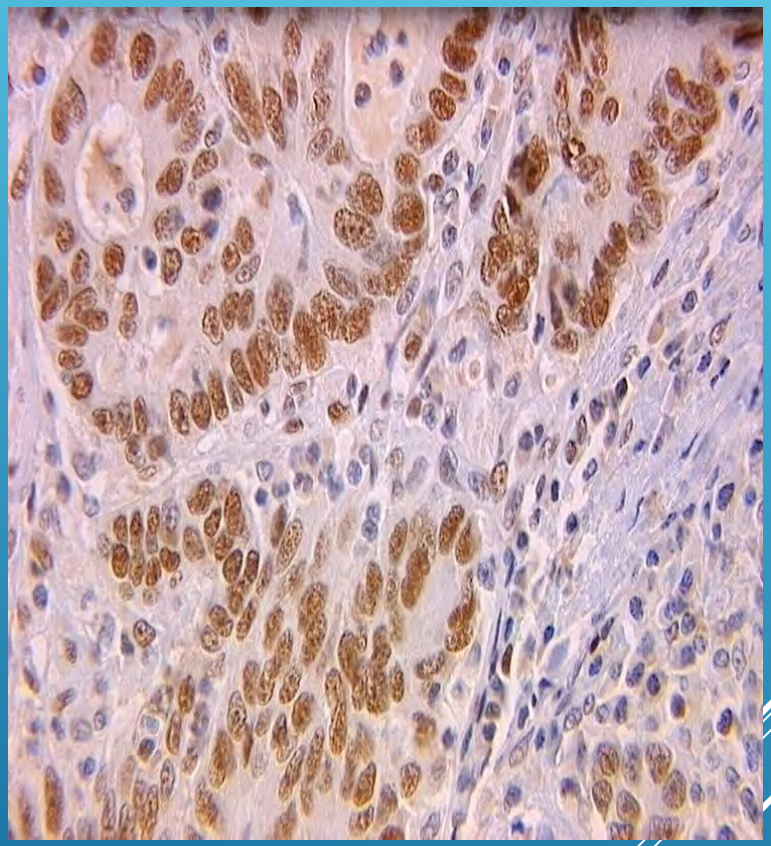


PMS2

GERMLINE *MLH1* MUTATION

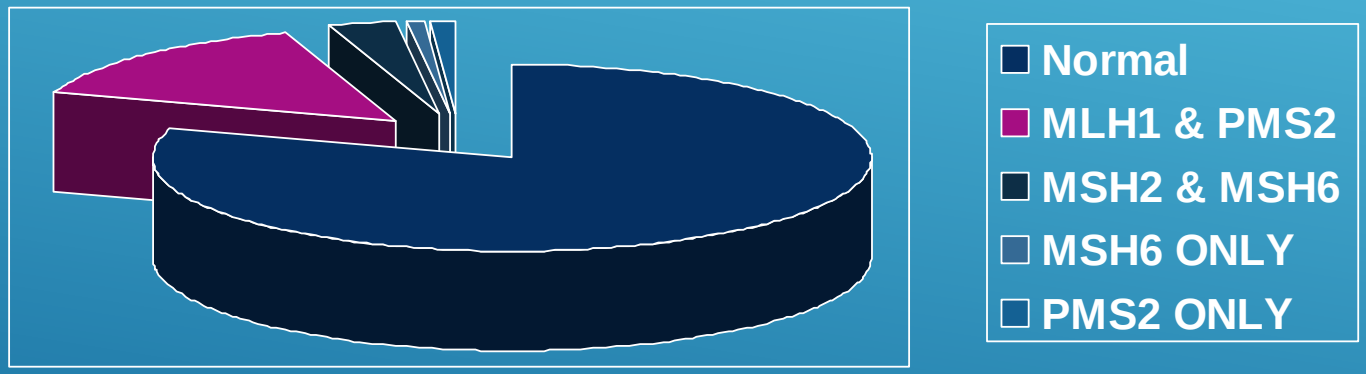


MLH1



MSH2

5 POSSIBLE RESULT FROM IHC TESTING



1. NORMAL – ALL 4 STAINS PRESENT

- 80% of cases
- CRC is probably not MSI+
- Prognosis worse than if MSI+
- Refer to Genetics if you have a patient with polyposis, diagnosed CRC <45, has had multiple adenomatous polyps or CRC primaries.

2. ABNORMAL – MLH1 & PMS2 ABSENT

- 15% of cases
- CRC is MSI+
- Better Prognosis
- 80% of these will be acquired methylation of *MLH1* and not HNPCC
- 20% will be HNPCC
- Refer for genetic testing

3. ABNORMAL – MSH2 & MSH6 ABSENT

- 3% of cases
- CRC is MSI+
- Better Prognosis
- Most likely HNPCC due to either *MSH2* or *MSH6* gene mutation
- Refer for genetic testing

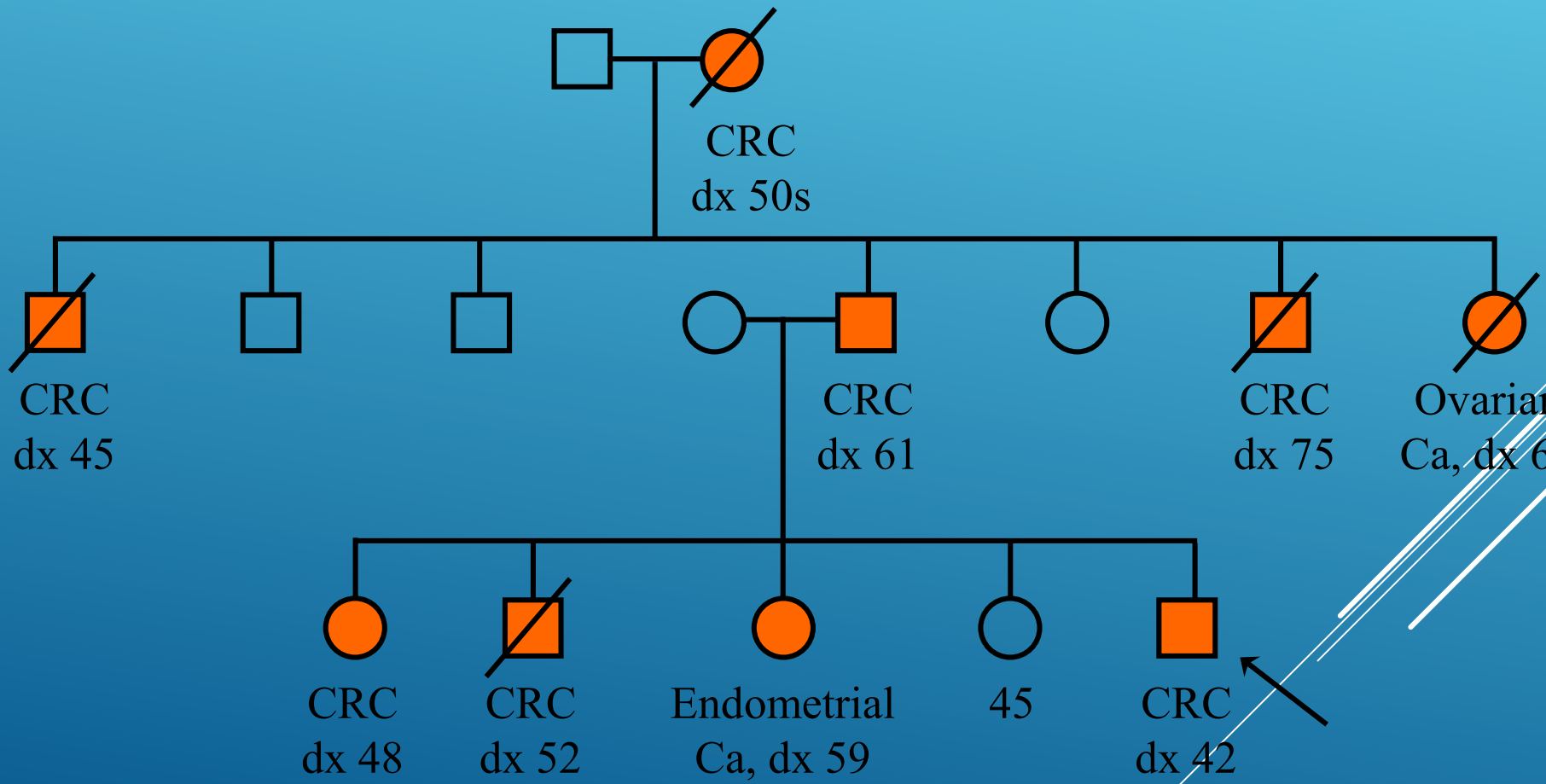
4. ABNORMAL – MSH6 ABSENT

- 1% of cases
- CRC is MSI+
- Better Prognosis
- Most likely HNPCC due to an *MSH6* gene mutation
- Refer for genetic testing

5. ABNORMAL – PMS2 ABSENT

- 1% of cases
- CRC is MSI+
- Better Prognosis
- Most likely HNPCC due to an *PMS2* gene mutation
- Refer for genetic testing

The Family History Is Key to Diagnosing HNPCC



Power of Partnership: Columbus wide study

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MAY 5, 2005 VOL. 352 NO. 18

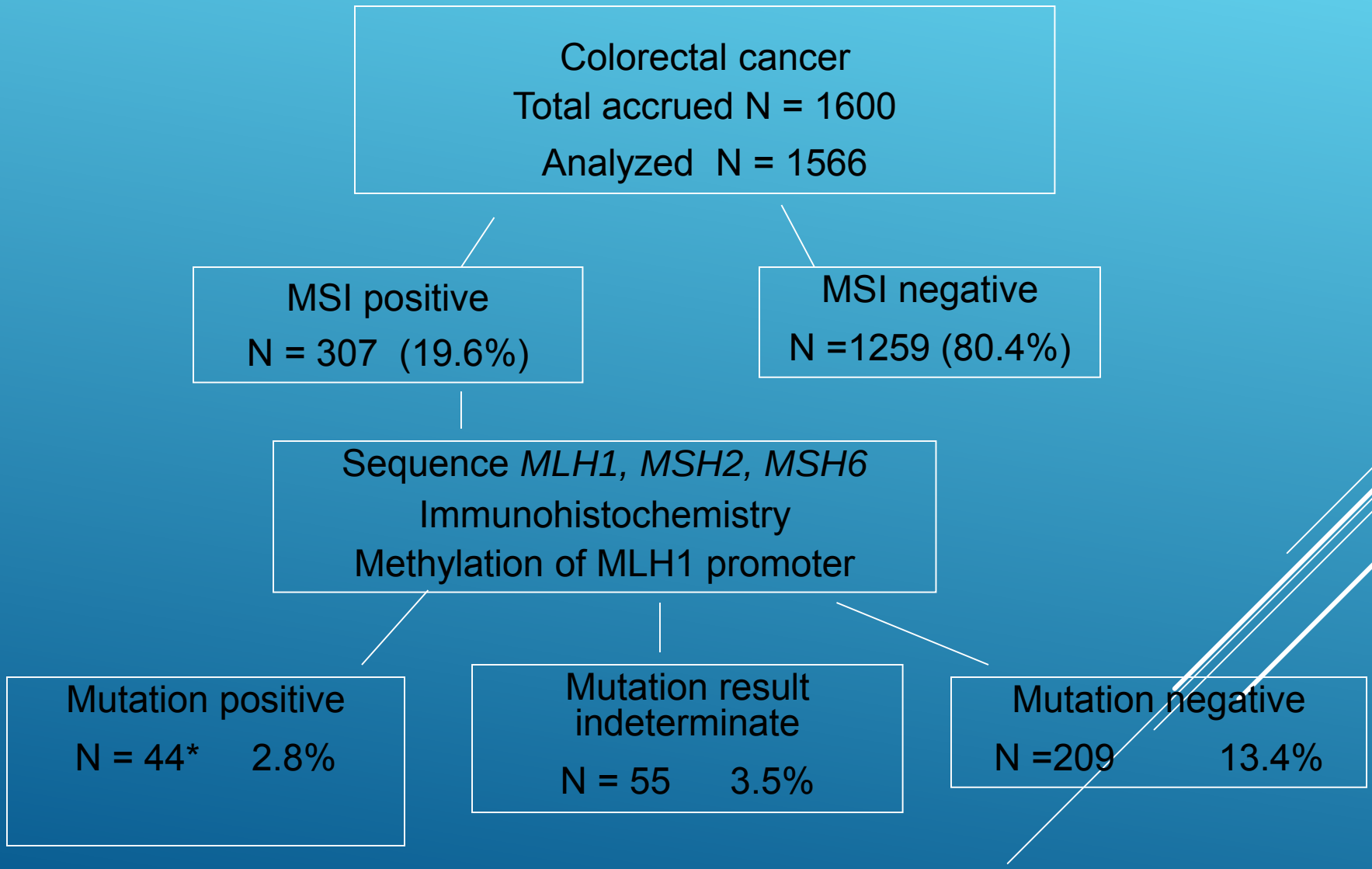
Screening for the Lynch Syndrome (Hereditary Nonpolyposis Colorectal Cancer)

Heather Hampel, M.S., Wendy L. Frankel, M.D., Edward Martin, M.D., Mark Arnold, M.D., Karamjit Khanduja, M.D., Philip Kuebler, M.D., Ph.D., Hidewaki Nakagawa, M.D., Ph.D., Kaisa Sotamaa, M.D., Thomas W. Prior, Ph.D., Judith Westman, M.D., Jenny Panescu, B.S., Dan Fix, B.S., Janet Lockman, B.S., Ilene Comeras, R.N., and Albert de la Chapelle, M.D., Ph.D.*

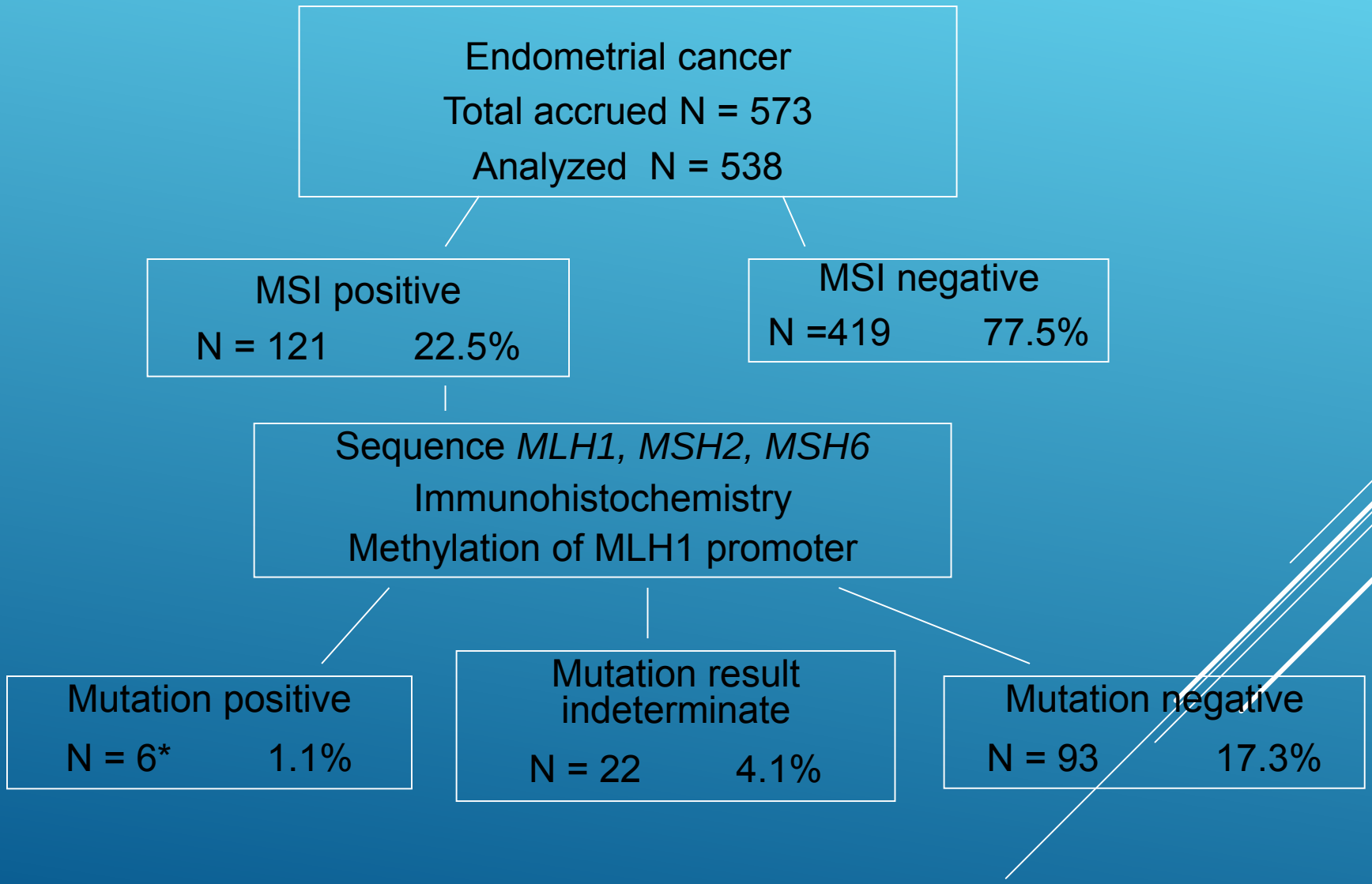
THE COLUMBUS-AREA HNPCC STUDY DESIGN

- Newly dx patients with CRC or EC enrolled regardless age/family history
- MSI+; IHC and mutation analysis of *MLH1*, *MSH2*, *MSH6* and *PMS2* genes by full sequencing of genomic DNA
  - Methylation status *MLH1* promoter evaluated by methylation-specific PCR and bisulfite-PCR followed by restriction digestion of tumor DNA

COLUMBUS HNPCC STUDY 1999-2005



Columbus HNPCC study



CRC HNPCC PROBAND CHARACTERISTICS

- Mean age at diagnosis – 50.4
  - Range, 23 to 87
- 39% CRC probands were not diagnosed <50
- 22% CRC probands did not meet Amsterdam or Bethesda criteria
- 57% MSH2 mutations, 22% MLH1 mutations, 13% MSH6 mutations, 8% PMS2 mutations

Columbus HNPCC study
Family studies of 33 probands

27 CRC & 6 ENDO probands recieved genetic counseling

Degree of Kinship	Tested	Positive
First	68	36
Second	30	9
> Second	44	18
Total	142	63

CONCLUSIONS

- Morbidity and mortality likely reduced by identifying probands and family members at risk and counseling
- In the Columbus area, the rate of MSI is 19% (CRC) and 23% (EC) and HNPCC is 2.2% (CRC) and 1.1% (EC)
- Large scale screening is feasible

SURVEILLANCE OPTIONS FOR CARRIERS OF HNPCC-ASSOCIATED MUTATIONS

Malignancy	Intervention	Recommendation
Colorectal cancer	Colonoscopy	Begin at age 20–25, repeat every 1–2 years
Endometrial cancer	Transvaginal ultrasound Endometrial aspirate	Annually, starting at age 25–35

Cancer Genetics Studies Consortium Task Force Recommendations
Modified from Burke W et al. *JAMA* 277:915, 1997

15-year prophylactic colonoscopic screening

22 HNPCC families identified; 252 asymptomatic individuals at 50% à priori risk offered screening

133 accepted (Mean age 38.1 yrs; 73 males, 60 females)

119 declined (Mean age 38.8 yrs; 59 males, 60 females

Screening by colonoscopy every 3 years

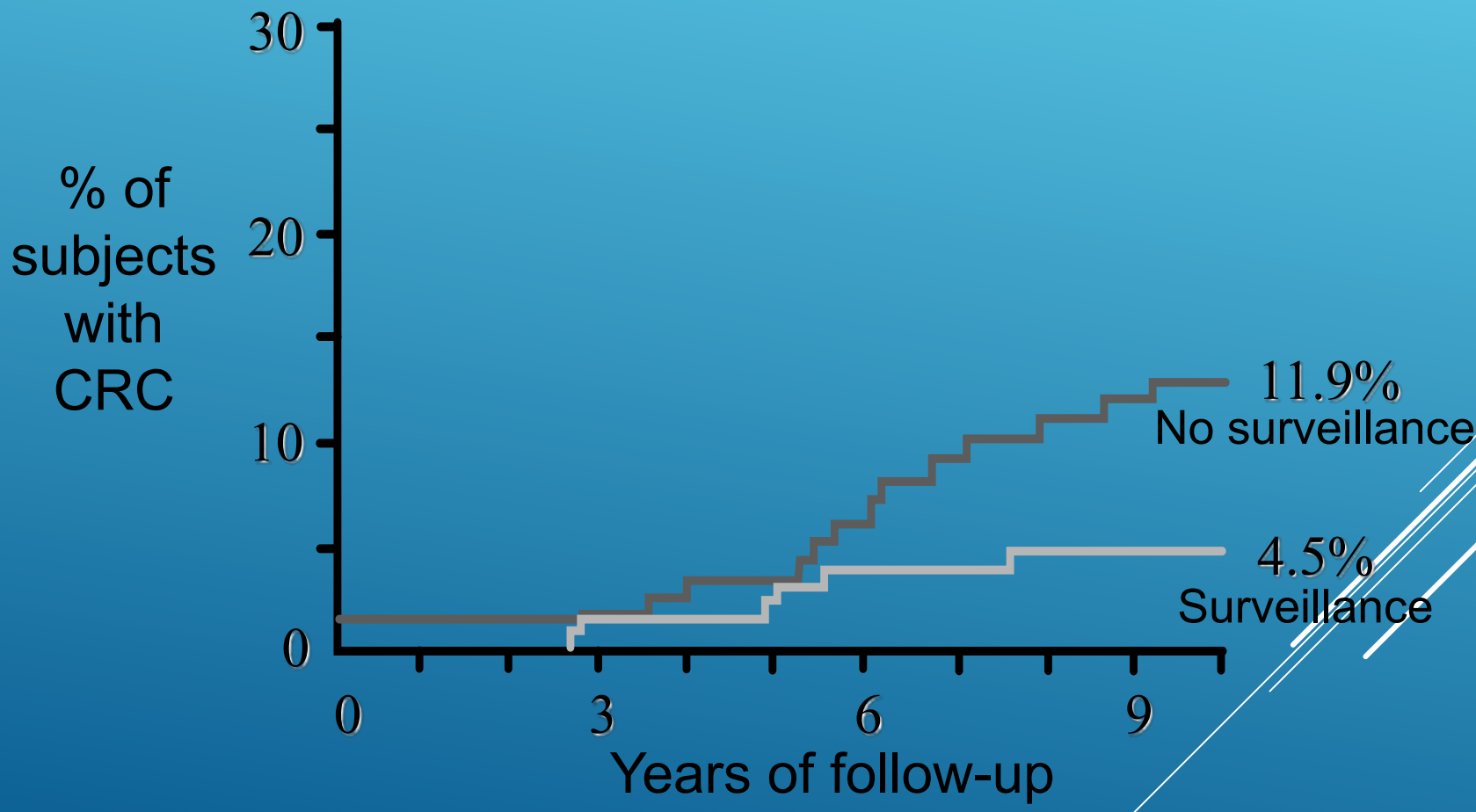
Järvinen et al. 1995 and 2000

15-year prophylactic colonoscopic screening

	Screened n=133	Not screened n=119	
Colorectal cancer	8	19	n=0.014
Death from colorectal cancer	0	9	p<0.001
Overall deaths	10	26	p<0.001

Järvinen et al. 1995 and 2000

SURVEILLANCE REDUCES RISK OF COLORECTAL CANCER IN HNPCC FAMILIES



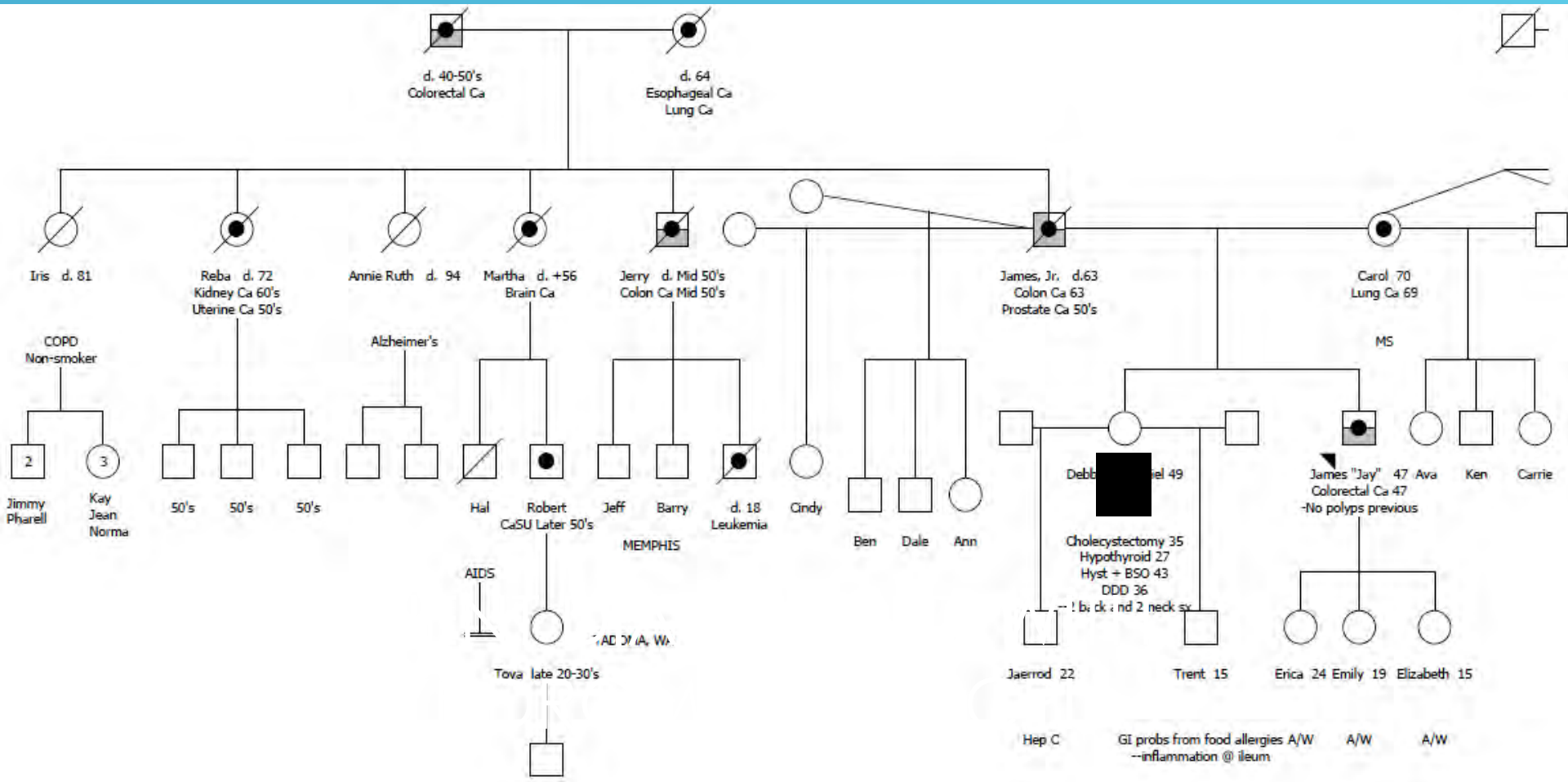
Jarvinen HJ et al. *Gastro* 108:1405, 1995

PROPHYLACTIC SURGERY FOR HNPCC-ASSOCIATED MUTATION CARRIERS

- Options include subtotal colectomy, hysterectomy, and oophorectomy
- Surgery does not eliminate cancer risk
- No recommendation for or against surgery has been made due to unproven efficacy

Burke et al. *JAMA* 277:915, 1997

Pedigree - HNPCC

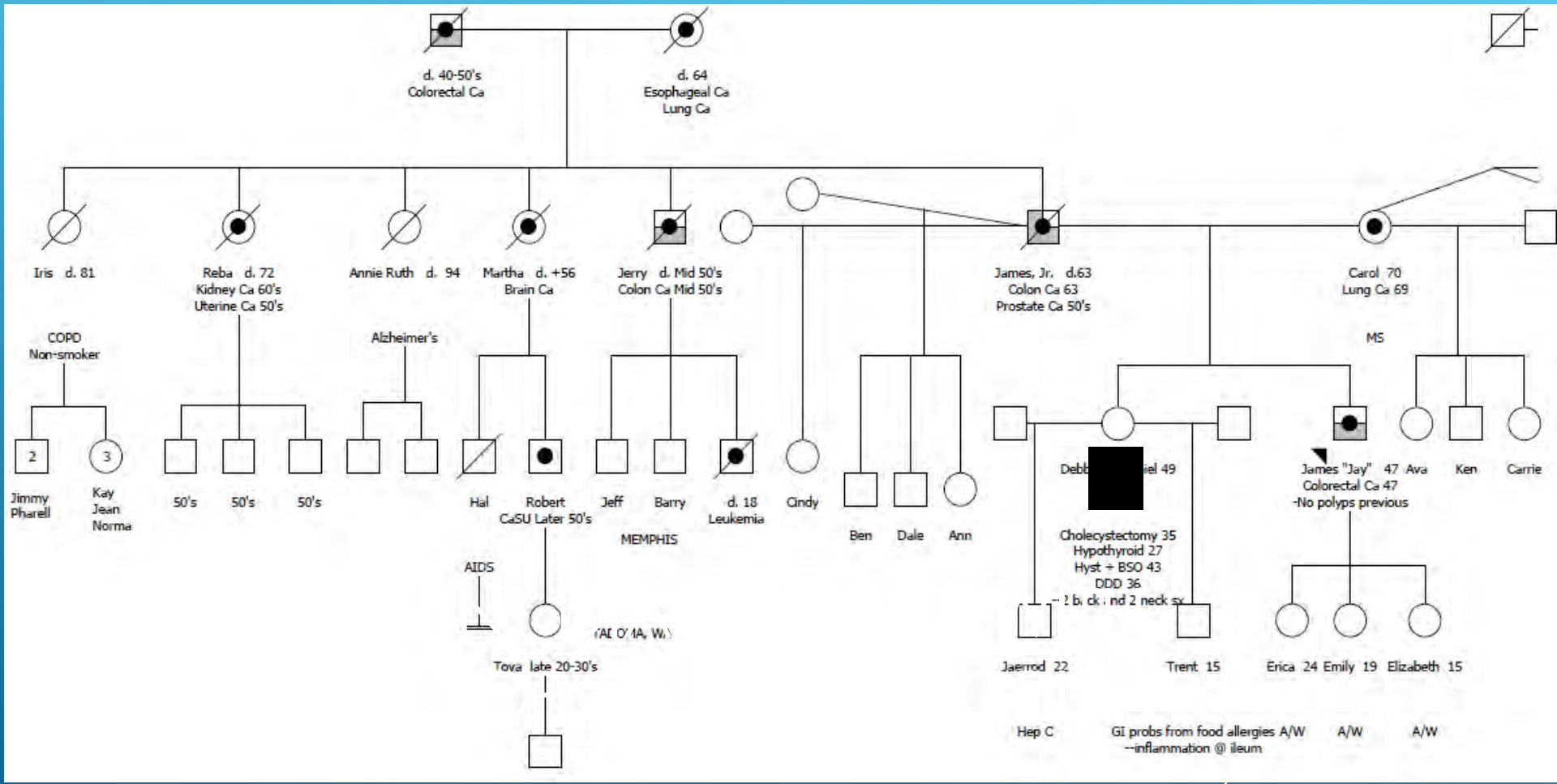


A LEGACY OF CANCER

Father

L to R: Uncle Jerry (colon), Grandma Ora Lee (lung), Martha (brain), Dad (colon) , Grandfather Jim Ben (colon), Aunt Reba (kidney & uterine cancer)

Pedigree - HNPCC



CASCADE TESTING

- Family Reunion June 2014
- On Mississippi River in western Kentucky
- Tested 20 at-risk relatives from his dad's side of the family
- Found one additional branch of family with Lynch syndrome
- They can now participate in cancer surveillance.

Lynch Syndrome Key Points

- All colon and rectal cancers should be screened for mismatch repair gene absence by IHC staining.
- The Amsterdam and Bethesda criteria are helpful aids in identifying patients at risk, but miss up to 30% of HNPCC families.
- It is desirable to identify mutation carriers, both affected and unaffected with cancer.
- Close follow-up and colonoscopy reduce the cancer risk.
- MSI+ tumors have better prognosis than MSI-.
- The benefits of aggressive surgery are unproven.