The pathology of inherited colorectal cancer syndromes

Marco Novelli
### Overview

- **Introduction**
- **Colorectal cancer pathways**
  - Chromosomal instability
  - Mismatch repair
  - Serrated
- **Colorectal cancer syndromes**
  - Inheritance
  - Types
Figure 1.3: Age-standardised incidence rates by sex, colorectal cancer, region of England, UK and Ireland, 1991-1999

Rate per 100,000

- Northern and... 
- Trent 
- West Midlands 
- North West 
- Eastern 
- London 
- South East 
- South West 
- England 
- Wales 
- Scotland 
- Northern Ireland 
- Ireland

UK and Ireland males 
UK and Ireland females

CANCER RESEARCH UK
Figure 1.2: Numbers of new cases and age-specific incidence rates, by sex, bowel cancer, UK 2006

- Male cases
- Female cases
- Male rates
- Female rates

Number of cases vs. Rate per 100,000 population vs. Age at diagnosis
Figure 1.2: Numbers of new cases and age-specific incidence rates, by sex, bowel cancer, UK 2006
## Genetics of CRC

- **Single gene**  
  - 5 - 10%

- **Complex genetic**  
  - 30%

- **“Sporadic”**  
  - 60%
Genetics of CRC

- Single gene: 5 - 10%
- Complex genetic: 30%
- “Sporadic”: 60%

‘Hereditary’ colorectal cancers / per annum
→ UK: 2000 – 4000
Colon cancer pathways
Table 4  Molecular classification of colorectal carcinoma

<table>
<thead>
<tr>
<th>Heredity</th>
<th>Chromosomal instability pathway</th>
<th>Mismatch repair pathway</th>
<th>Serrated pathway</th>
<th>Hybrid pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hereditary and sporadic</td>
<td>Hereditary</td>
<td>Hereditary and sporadic</td>
<td>Sporadic</td>
</tr>
<tr>
<td>CIMP status</td>
<td>Negative</td>
<td>Negative</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>MSI status</td>
<td>MSS</td>
<td>MSI-H</td>
<td>MSI-H</td>
<td>MSI-L or MSS</td>
</tr>
<tr>
<td>Chromosomal instability</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>KRAS mutation</td>
<td>+++</td>
<td>+/-</td>
<td>---</td>
<td>+++</td>
</tr>
<tr>
<td>BRAF mutation</td>
<td>---</td>
<td>---</td>
<td>+++</td>
<td>---</td>
</tr>
<tr>
<td>MLH1 status</td>
<td>Normal</td>
<td>Mutation</td>
<td>Methylated</td>
<td>Partial methylation</td>
</tr>
<tr>
<td>MGMT methylation</td>
<td>---</td>
<td>---</td>
<td>+/-</td>
<td>+++</td>
</tr>
</tbody>
</table>

Abbreviations: CIMP, CpG island methylator phenotype; MGMT, O-6-methylguanine DNA methyltransferase; MSI, microsatellite instability; MSI-H, high-level microsatellite instability; MSI-L, low-level microsatellite instability; MSS, microsatellite stability.

Serrated polyps and colorectal cancer: new pathway to malignancy. Noffsinger AE.

Annu Rev Pathol. 2009;4:343-64.
Serrated polyps and colorectal cancer: new pathway to malignancy. Noffsinger AE.

Annu Rev Pathol. 2009;4:343-64.
Main epidermal growth factor receptor signaling pathways: PI3K/pAKT pathway (blue); RAS/RAF/mitogen-activated protein kinase pathway (red).

Loupakis F et al. JCO 2009;27:2622-2629
Many mutations make protein constitutively active

→ Proteins turned on all the time

→ Blocking EGFR becomes ineffective.
Pathways in sporadic CRC

- Chromosomal instability $\approx 70\%$.
- CIMP (CpG island methylator) $\approx 30\%$. 
Pathways in sporadic CRC

- Chromosomal instability $\approx 70\%$.
- CIMP (CpG island methylator) $\approx 30\%$.

- $\approx 25\%$ of MSI cancers can exhibit chromosomal abnormalities.
- $\approx 33\%$ of CIMP+ve tumours exhibit chromosomal abnormalities.
- $\approx 12\%$ CIN cancers exhibit high levels of MSI.
Pathways implicated in colorectal tumorigenesis

- Wnt/β-catenin
- TGF-β/SMAD
- EGFR-
  - RAS/RAF/MAPK cascade
  - PI3K/Akt pathway
- Notch
- Hedgehog
Pathways implicated in colorectal tumorigenesis

- Wnt/β-catenin
- TGF-β/SMAD
- EGFR-
  - RAS/RAF/MAPK cascade
  - PI3K/Akt pathway
- Notch
- Hedgehog

- Cell proliferation
- Apoptosis
- Differentiation
- Cell migration
Colorectal carcinogenesis

- Multiple pathways for colorectal carcinogenesis and they are not mutually exclusive.
- Individual pathways can be targeted at different points e.g. KRAS/BRAF.
- Not just accumulation of mutations, but order of mutation acquisition may be important.
Colorectal Cancer Syndromes

- Lynch Syndrome (HNPCC)
- Familial Adenomatous Polyposis
- MYH-associated polyposis
- Hyperplastic polyposis
- Mixed Polyposis
- Polymerase Proof reading-associated polyposis (PPAP)
- Peutz Jeghers
- Juvenile polyposis
- Childhood cancer syndrome
Mendelian inheritance
Mendelian Autosomal Dominant Inheritance

50% Normal

50% Affected
Autosomal Dominant Inheritance

FIG. 89-1. Autosomal-dominant inheritance. Vertical pattern of trait expression; males and females are equally affected.
Mendelian Autosomal Recessive Inheritance

- 25% Normal
- 50% Heterozygote
- 25% Affected
Autosomal Recessive Inheritance

FIG. 89-2. Autosomal recessive inheritance. Horizontal pattern of trait expression. Parental consanguinity is often present in families with autosomal recessive disorders.
Colorectal Cancer Syndromes

• Lynch Syndrome (HNPCC)
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Colorectal Cancer Syndromes

• Lynch Syndrome (HNPCC) Dominant
• Familial Adenomatous Polyposis Dominant
• MYH-associated polyposis Recessive
• Hyperplastic polyposis Unknown
• Mixed Polyposis Dominant
• Polymerase Proof reading-associated polyposis (PPAP) Dominant
• Peutz Jeghers Dominant
• Juvenile polyposis Dominant
• Childhood cancer syndrome Recessive
Lynch syndrome (HNPCC)

- Most common form of familial CRC (?3% CRC).
- Autosomal dominant inheritance.
- Mutation in mismatch repair gene (MMR)
  - Family of DNA repair genes.
    - MLH1, MSH2, MSH6 + PMS2.
- Usually inherited, but may be new mutation.
Lynch syndrome

Clinical features:

- Colorectal adenocarcinomas
  - synchronous/metachronous tumours in 30%.

- Tumours at other sites:
  - Endometrium, stomach, small bowel, urinary tract, pancreatobiliary tract, brain, skin.

- Early age of tumour development
  - Typically in 40s and 50s.
Features of typical HNPCC colorectal adenocarcinomas

- Right-sided.
- Poorly differentiated.
- Mucinous differentiation.
- Prominent inflammatory response.
### Criteria used by clinical geneticists

**Table 1 Amsterdam criteria I [21]**

<table>
<thead>
<tr>
<th>Families must fulfil all criteria:</th>
</tr>
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<tbody>
<tr>
<td>1. There should be at least three relatives with a CRC.</td>
</tr>
<tr>
<td>2. One should be a first-degree relative of the other two.</td>
</tr>
<tr>
<td>3. At least two successive generations should be affected.</td>
</tr>
<tr>
<td>4. At least one should be diagnosed before the age of 50 years.</td>
</tr>
<tr>
<td>5. Familial adenomatous polyposis (FAP) should be excluded.</td>
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<td>6. Tumours should be verified by pathological examination.</td>
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**Table 2 Amsterdam criteria II [23]**

<table>
<thead>
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<th>Families must fulfil all criteria:</th>
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<tr>
<td>1. There should be at least three relatives with a LS-associated cancer*.</td>
</tr>
<tr>
<td>2. One should be a first-degree relative of the other two.</td>
</tr>
<tr>
<td>3. At least two successive generations should be affected.</td>
</tr>
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<td>4. At least one should be diagnosed before the age of 50 years.</td>
</tr>
<tr>
<td>5. Familial adenomatous polyposis (FAP) should be excluded in the CRC case(s), if any.</td>
</tr>
<tr>
<td>6. Tumours should be verified by pathological examination.</td>
</tr>
</tbody>
</table>

**Table 3 Original Bethesda Guidelines [28]**

<table>
<thead>
<tr>
<th>Individuals meeting any one of the following should undergo MSI testing:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Individuals with cancer in families that meet the Amsterdam criteria.</td>
</tr>
<tr>
<td>2. Individuals with two LS-related cancers, including synchronous and metachronous CRCs or associated extracolonic cancers*.</td>
</tr>
<tr>
<td>3. Individuals with CRC and a first-degree relative with CRC and/or LS-related extracolonic cancer and/or a colorectal adenoma; one of the cancers diagnosed at age &lt;45 years, and the adenoma diagnosed at age &lt;40 years.</td>
</tr>
<tr>
<td>4. Individuals with CRC or endometrial cancer diagnosed at age &lt;45 years.</td>
</tr>
<tr>
<td>5. Individuals with right-sided CRC with an undifferentiated pattern (solid/cribriform) on histopathology diagnosed at age &lt;45 years ‡.</td>
</tr>
<tr>
<td>6. Individuals with signet-ring-cell-type CRC (more than 50% signet ring cells) diagnosed at age &lt;45 years.</td>
</tr>
<tr>
<td>7. Individuals with adenomas diagnosed at age &lt;40 years.</td>
</tr>
</tbody>
</table>

*Endometrial, ovarian, gastric, hepatobiliary or small-bowel cancer or transitional cell carcinoma of the renal pelvis or ureter.

‡Solid/cribriform defined as poorly differentiated or undifferentiated carcinoma composed of irregular, solid sheets of large eosinophilic cells and containing small gland-like spaces.
Table 5 Dutch guideline for MSI testing (www.oncoline.nl)

The pathologist is advised to requests MSI testing (and immunohistochemistry of the MMR proteins) in the following patients:

1. CRC or endometrial carcinoma before the age of 50 years.

2. A second CRC before the age of 70 years.

3. CRC before the age of 70 years AND another synchronous or previous LS-associated tumour*.

*CRC, endometrial, ovarian, gastric, small bowel, pancreas, hepatobiliary tract, renal pelvis or ureter, and brain tumours, sebaceous gland adenomas and keratoacanthomas.
Is it a case of Lynch syndrome?

- Age of patient
- Site of tumour
- Tumour multiplicity
- Histological features of tumour
- Family history
Is it case of Lynch syndrome?

- Age of patient
- Site of tumour
- Tumour multiplicity
- Histological features of tumour
- Family history

Low index of suspicion

- Immunostaining for 4 mismatch repair genes.
- Consider referral to a clinical geneticist.
Mismatch repair immunostaining
A. Initiation

- MLH1
- PMS2
- MSH2
- MSH6

B. Excision & resynthesis

- Exol
- MSH2
- MSH3
- Pol δ/ε
- PCNA
- DNA ligase I
- PMS2
- MLH1
Loss MLH1 (mutation or methylation)

Loss PMS2

Loss MSH2

Loss MSH6
Loss of MLH1/PMS2 → MLH1 mutation or promoter hypermethylation
Loss of MSH2/MSH6 → MSH2 mutation
Loss of MSH6 expression → MSH6 mutation
Loss of PMS2 expression → PMS2 mutation
Familial Adenomatous Polyposis
Familial adenomatous polyposis (FAP)

> 100 colorectal polyps
Familial adenomatous polyposis

- > 100 polyps predominantly in large intestine (typically 1000s).

- Colorectal adenocarcinomas < 40 years old (10-15 years after appearance of polyps).

- Extra-intestinal manifestations.

- Commonest cause death now duodenal carcinoma and desmoids.

- Due to a germline mutation in the \( Apc \) gene (5q22)
Congenital Hyperpigmentation of the Retinal Pigment Epithelium (CHRPE).

- Normal population 5% (0.3 – 40%)
- FAP 66-92%
CHRPE in FAP are typically bilateral with a de-pigmented halo.
Fundic gland polyps:

- 0.8–2% normal population.
- 12.5–84% FAP patients
Desmoid tumours

- Rare tumours 2-4/million per annum
- 10% (3.5 – 32%) incidence in FAP
APC protein domains and FAP phenotype association with (truncating) germline mutation position

Heptad repeats (dimerisation)

15-amino acid repeats (β-catenin binding)

NESR3

SAMP1

Basic domain (Microtubule binding, tubulin polymerisation)

Armadillo repeats

20-amino acid repeats (β-catenin binding, GSK3 β phosphorylation)

EB1 and HDLG binding sites

Classical FAP

Severe FAP

AAPC

CHRPE

Desmoids

6 57 453 767 1020 1169 1262 2033 2000 2200 2400 2560 2843

168 1580

1250 1309 1464

78 400 1580

457 1444

1395 2000
Attenuated FAP

- Typically less than 100 adenomas in colon.
- Mean 25 adenomas (0 - 470).
- Concentrated in proximal colon.
- Some extra-colonic manifestations.
- Colorectal carcinoma in 70% by 80 years of age.
MYH-associated polyposis (MAP)
Discovery of MYH-associated polyposis

- Family with multiple adenomas and CRC
- No known pathogenic APC or MMR germline mutation
- 3 affected sibs - 11 tumours
  - 15/18 somatic APC mutations G:C>T:A

→ Suggested mutations from oxidative DNA damage.

Al-Tassan *et al* Nat Genet 30:227-232, 2002
Oxidative damage of DNA

• Oxidative damage - $10^4$ lesions/cell/day.
• 8-oxo-G most deleterious.
• 8-oxo-G mis-pairs with adenine residues

<table>
<thead>
<tr>
<th>Normal G:C</th>
<th>Damaged $G^o:A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>G:C → T:A</td>
<td>mutation</td>
</tr>
</tbody>
</table>

• Role of MYH is to excise mispaired adenines (Base excision repair BER).
Base Excision Repair

Oxidative damage

OGG1

BER

MYH
Oxidative damage

OGG1

BER

MYH Dysfunction

MYH
Oxidative damage

MYH-associated polyposis

OGG1

BER

MYH Dysfunction

MYH

G:C → T:A transversion
MYH-associated polyposis

- Autosomal recessive inheritance.
- Penetrance approaching 100%.
- Clinically similar to attenuated FAP.
  - Typically <100 polyps (but may show classical FAP phenotype).
  - Some extra colonic manifestations.
  - May also have serrated polyps
- Probably accounts for up to 30% of FAP cases with no documented APC mutation.
MAP families - AR inheritance
MYH-associated Polyposis

- Due to biallelic mutations in MYH gene.
- Thought that there is a gradual accumulation oxidative damage causing tumorigenesis
  - \( APC \rightarrow \) classical adenomas
  - \( KRAS \rightarrow \) serrated polyps
- Mean age cancer diagnosis 50 years old.
- Up to 30% of European patients with > 15 - 100 adenomas carry biallelic MYH mutations.
MYH-ASSOCIATED NEOPLASIA

There are several clinical situations that suggest MAN.

1. Patient with apparent FAP based on finding more than 100 synchronous colorectal adenomas, with or without a family history, in whom APC mutation testing is negative.

2. Patient with multiple (>10 cumulative, but <100) adenomas where FAP is unlikely, especially where the family history suggests recessive inheritance.

3. Young (<50 years) age-of-onset colorectal cancer, with or without a family history of colorectal neoplasia.

4. Familial clustering of colorectal cancer that does not fulfill Amsterdam criteria for HNPCC, where tumors are microsatellite stable.

5. HNPCC by Amsterdam criteria with microsatellite stable tumors (familial colorectal cancer type X).

6. HNPCC by Amsterdam criteria with microsatellite unstable tumors but no mismatch repair gene mutation.

7. A “mixed polyposis” of adenomas and serrated polyps.

Suspected MAP and attenuated FAP patients

- Consider referral to geneticist.
- Mutation analysis - Germline analysis on blood

**MAP**
- p.Tyr165Cys and p.Gly382Asp account for 85% of MAP mutations in Cauc Hans.
- Initial analysis PCR-based.
- If PCR negative then full gene sequencing.

**FAP**
- gene sequencing

- Best practice guidelines: Clinical Molecular Genetics Society

- London – Kennedy Galton Centre
  http://www.nwlh.nhs.uk/nwthamesgenetics
Hereditary mixed polyposis syndrome (CRAC1)

- Ashkenazi Jewish origin.
- Variety of colonic tumours.
  - Atypical juvenile polyps
  - Serrated adenomas
  - Hyperplastic polyps
  - Classical adenomas
  - Carcinomas
- CRAC1 locus 15q13.3-14.
- Possible to screen family members ("ancestral haplotype"), but cannot directly test for gene.
Polymerase Proofreading-associated polyposis (PPAP)

- Germline POLE and POLD1 mutations.
- Major catalytic and proofreading subunits of the Polε and Polδ enzyme complexes
- Mutations impair proofreading → hypermutation
Polymerase Proofreading-associated polyposis (PPAP)

- Autosomal dominant with high penetrance.
- Multiple colorectal adenomas, early-onset CRC, multiple CRCs and endometrial cancers.
- Conventional adenomas and adenocarcinomas (no specific histological features).
- Tumours microsatellite stable but have multiple point mutations.
- POLE and POLD1 also mutated in sporadic CRC.

Hyperplastic polyposis
WHO diagnostic criteria for hyperplastic polyposis.

- At least five histologically diagnosed hyperplastic (serrated) polyps proximal to the sigmoid colon of which two are greater than 10mm in diameter.
  
  or

- Any number of hyperplastic polyps occurring proximal to the sigmoid colon in an individual with a first-degree relative with hyperplastic polyposis.
  
  or

- More than 30 hyperplastic polyps of any size distributed throughout the colon.
Hyperplastic polyposis

- Genetics poorly understood.

- Develop multiple serrated polyps:
  - Hyperplastic polyps,
  - Sessile serrated polyps/adenomas
  - Classical serrated adenomas.

- Up to 50% may develop colorectal cancer.

- Risk of synchronous/metachronous carcinomas.
Hyperplastic polyp

- Usually < 5mm diameter.
- Usually left-sided (sigmoid + rectum).
- Usually middle to old age (mean 62 years)
Sessile serrated polyp/adenoma

- Usually right-sided
- Large (typically > 10mm)
Sessile serrated polyp/adenoma

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Major histologic features of sessile serrated polyps</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Abnormal proliferation/dysmaturation</td>
</tr>
<tr>
<td></td>
<td>Nuclear atypia in middle/upper crypts</td>
</tr>
<tr>
<td></td>
<td>Oval nuclei in middle crypts</td>
</tr>
<tr>
<td></td>
<td>Prominent nucleoli in middle/superficial crypts</td>
</tr>
<tr>
<td></td>
<td>Dystrophic goblet cells</td>
</tr>
<tr>
<td></td>
<td>Irregular distribution of goblet cells</td>
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<td></td>
<td>Mitoses in middle/upper crypts</td>
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<tr>
<td></td>
<td>Architectural abnormalities</td>
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<tr>
<td></td>
<td>Basal crypt dilation</td>
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<tr>
<td></td>
<td>Horizontal orientation of deep crypts</td>
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<tr>
<td></td>
<td>Prominent serrations</td>
</tr>
<tr>
<td></td>
<td>Serration to base of crypt</td>
</tr>
<tr>
<td></td>
<td>Inverted crypts</td>
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<tr>
<td></td>
<td>Other features</td>
</tr>
<tr>
<td></td>
<td>Lack of thickened basement membrane</td>
</tr>
<tr>
<td></td>
<td>Focal loss of $MLH1$ positivity</td>
</tr>
</tbody>
</table>
Sessile serrated lesion

- Risk of developing high grade dysplasia and carcinoma within the polyp.
  → should be removed where possible.
- Sessile serrated polyps/adenomas are said to be associated with synchronous advanced colorectal neoplasia.
- Sessile serrated polyps/adenomas raise the possibility of hyperplastic polyposis.
Peutz-Jeghers syndrome
Peutz-Jegher’s polyps
Peutz-Jeghers syndrome

- Autosomal dominant inheritance.
- Gene LKB1 (p53-mediated apoptosis)
- 39% lifetime risk CRC.
- 93% risk of developing malignancy by age 65.
  - Carcinomas of colorectum, pancreas, stomach, small intestine, oesophagus.
  - Breast, ovary, endometrium and lung cancers.
  - Melanoma and sex cord tumours.
• Searched pathology database over 22 years.
• 121 PJ polyps diagnosed (from 38 patients).
• 102 PJ polyps after review.
  – 94 polyps in patients with PJS
  – 8 remaining polyps.
    • 3 patients probable PJS.
    • 5 colonic polyp with features suspicious of PJ (4/5 patients had history/FH of tumours).
Searched pathology database over 22 years.

121 PJ polyps diagnosed (from 38 patients).

102 PJ polyps after review.
- 94 polyps in patients with PJS
- 8 remaining polyps.
  - 3 patients probable PJS.
  - 5 colonic polyp with features suspicious of PJ (4/5 patients had history/FH of tumours).

If they exist sporadic PJ polyps are very rare.

Individuals with solitary PJ polyp have a high risk of developing malignancy.

14% misdiagnosed
Juvenile Polyposis Syndrome (JPS)
Juvenile polyposis

Diagnostic criteria:

- > 3-5 Juvenile polyps in colorectum.
  
  or

- Juvenile polyps throughout GI tract.
  
  or

- Juvenile polyp + family history.
Juvenile Polyposis Syndrome (JPS)

- Autosomal dominant.
- Hamartomatous polyps of the GI tract.
- Colorectal, gastric, duodenal and pancreatic carcinomas
- Up to 70% lifetime risk CRC
- Median age diagnosis CRC 42 years
- SMAD4 – $\approx 20\%$ (TGF$\beta$ signaling)
- BMPR1A – $\approx 20\%$ (TGF$\beta$ signaling)
Childhood cancer syndrome
(Congenital Mismatch Repair Deficiency)
• “Autosomal recessive” HNPCC.
• Biallelic mutations in PMS2, MSH2, MSH6 or MLH1 (compound or homozygous).

• Phenotype:
  – Café-au-lait spots (NF1 type features).
  – Colorectal cancers and adenomas.
  – Brain tumours (Gliomas, PNETs, medulloblastomas).
  – Haematological malignancies (leukaemias and diffuse large B cell lymphoma).

• Accounts for some cases of Turcot syndrome.
Childhood cancer syndrome

- Autosomal recessive inheritance
Childhood cancer syndrome

- Most commonly seen in Asian and Arabic populations where consanguinous marriages are common
• 9 year old male
• Café-au-lait spots.
• Multiple colorectal carcinomas/adenomas.
• Glioblastoma multiforme
Rectal adenocarcinoma
Glioblastoma multiforme
→ No PMS2 staining in tumour or normal tissues!?
CHILHOOD CANCER SYNDROME

• Young children

• Combination of:
  – Colorectal carcinoma
  – Café-au-lait spots.
  – Brain/haematological malignancies.

•Mismatch repair immunostaining may be completely negative or normal.
Conclusions

• The molecular biology of colorectal carcinoma is complex.

• Consider mismatch repair protein immunostaining in CRC patients <50 years of age.

• Consider MAP in patients with polyposis but no family history.

• Consider hyperplastic polyposis in patients with multiple serrated type lesions (For screening such patients use dye spray where possible).