GISCoR
gruppo italiano screening colorettale

XII CONGRESSO NAZIONALE 2017
7-8 Novembre 2017

CORSO PRE-CONGRESSO
7 Novembre 2017

Il rischio eredo-familiare del tumore colorettale: le predisposizioni ereditarie

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Why do we need CRC genetics?

- Cancer is a genetic disease of somatic cells.

  Gene asset may → dictate prognosis

  → guide "target therapy"

- A fraction of cancers occurs due to gene defect transmitted as germ-line mutation(s)

  = inherited predispositions

- Inherited gene defects variably predispose to CRC with different phenotypes (or no phenotype!)

Inherited GI cancer predisposition
Historical perspective

Intuition | Model | Biological bases & heterogeneity

1895-1913
Seed concept of predisposition

1930-40s
Description of polyposis coli

1970s
Lynch systematic review of predispositions

1990s
Molecular genetics identifies culprits

...

Evolution of the Concept of Inherited Predisposition

- **The phenotype era**
  - Recognition of predisposition to CRC as a mendelian trait -> *familial adenomatous polyposis* (FAP)
  - Cancer without polyposis -> Hereditary Non-Polyposis Colorectal Cancer (HNPCC) & Lynch Syndrome
- **The genotype era**
  - FAP= Adenomatous Polyposis Coli (*APC*) + *MUTYH*
  - Lynch = DNA Mismatch Repair System and its components (*MLH1, MSH2, MSH6, PMS2*)
  - The new candidates genes: expanding molecular genetics by next generation sequencing (**NGS**)

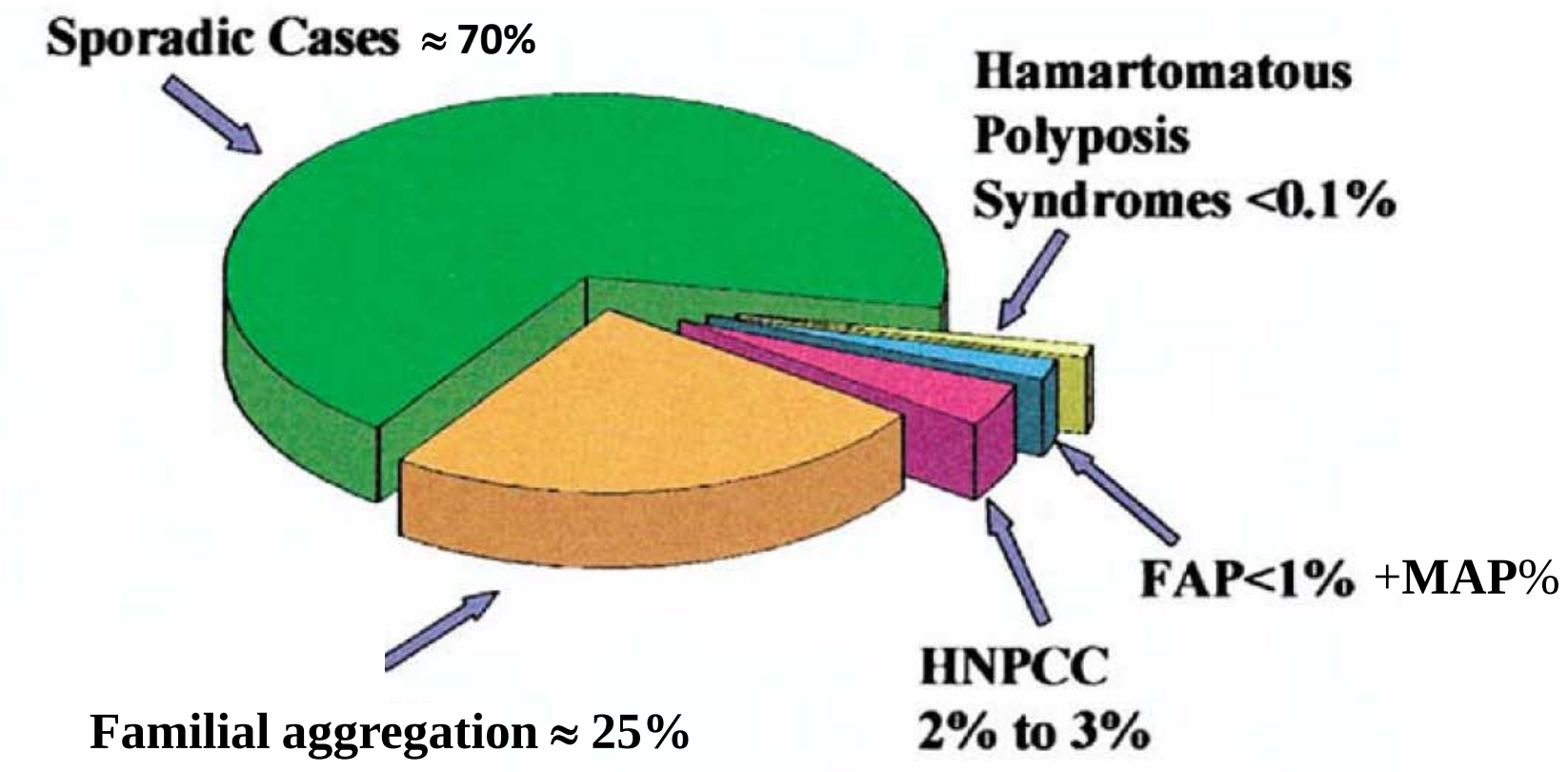
Hereditary GI Cancer

- **The main playground: CRC**
  - Polyposis in all its variants
  - Lynch syndrome
  - X-syndrome
  - the unknown & the poorly explored -> **NGS**

- Other territories
  - Pancreatic cancer
  - Gastric cancer

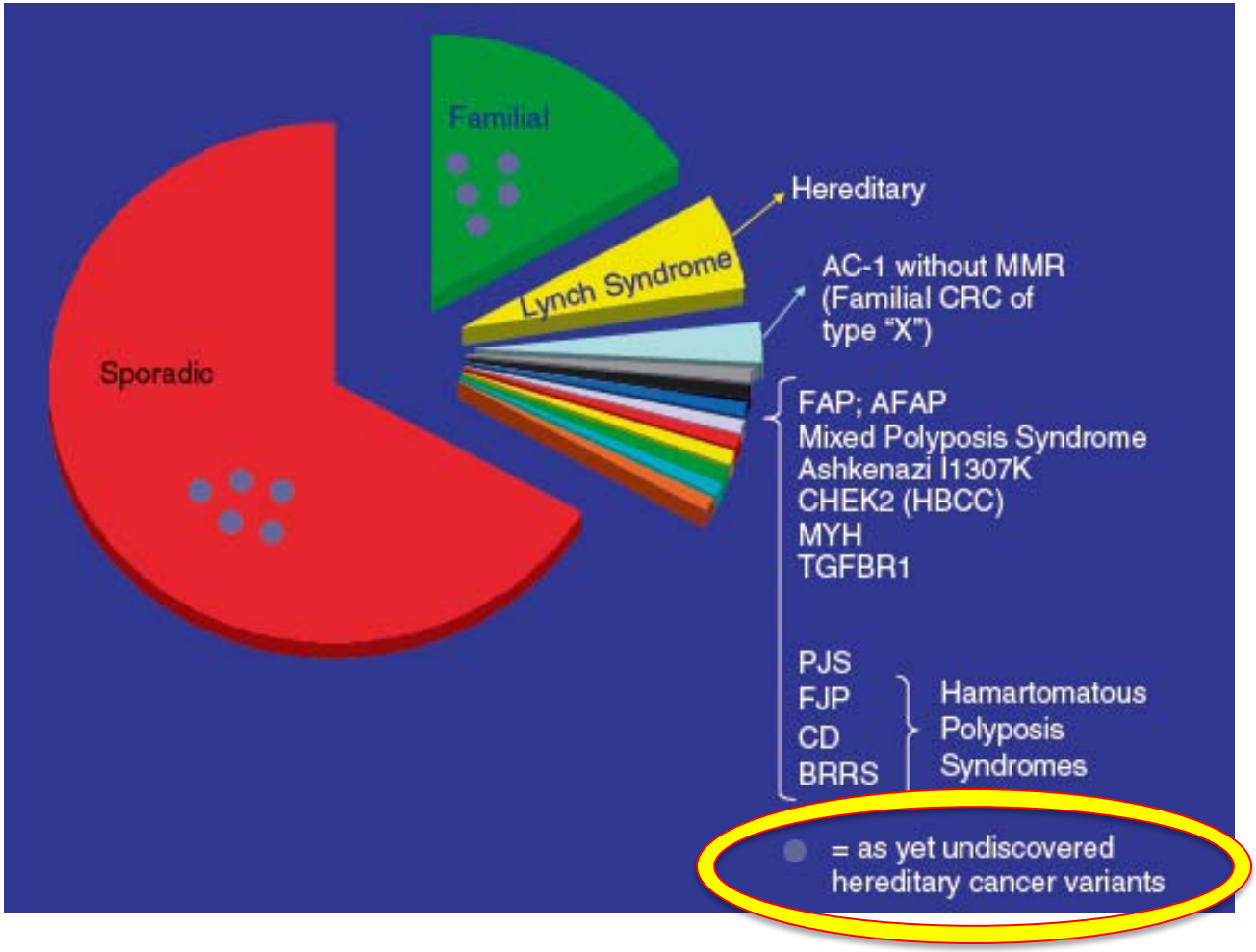
The school-days - childhood

Familial aggregation ≠ inheritance



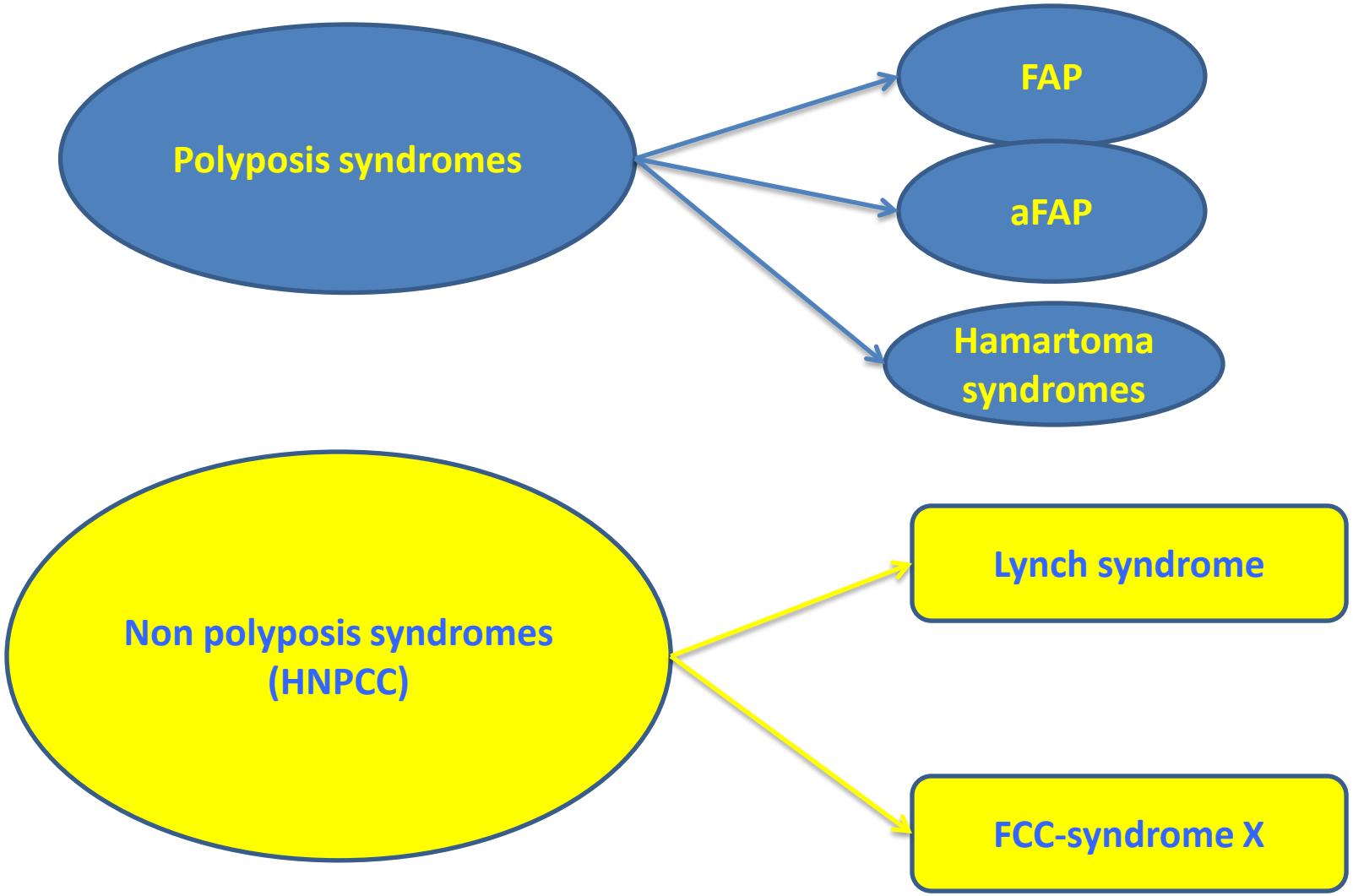
Burt, *Gastroenterology,* 2005

How changed the landscape of inherited predispositions to CRC



Boland, *Clin Genetics*, 2009

The familial CRC syndromes



from Boland, 2015

Complexity of inherited predispositions to CRC
The grocery list

Lynch syndrome (LS)
All newly diagnosed colorectal cancers (CRCs) should be evaluated for mismatch repair deficiency.
Analysis may be done by immunohistochemical testing for the *MLH1/MSH2/MSH6/PMS2* proteins and/or testing for microsatellite instability. Tumors that demonstrate loss of *MLH1* should undergo BRAF testing or analysis for *MLH1* promoter hypermethylation.
Individuals who have a personal history of a tumor showing evidence of mismatch repair deficiency (and no demonstrated BRAF mutation or hypermethylation of *MLH1*), a known family mutation associated with LS, or a risk of ≥5% chance of LS based on risk prediction models should undergo genetic evaluation for LS.
Genetic testing of patients with suspected LS should include germline mutation genetic testing for the *MLH1, MSH2, MSH6, PMS2,* and/or *EPCAM* genes or the altered gene(s) indicated by immunohistochemical (IHC) testing.
Adenomatous polyposis syndromes
Familial adenomatous polyposis (FAP)/MUTYH-associated polyposis/attenuated polyposis
Individuals who have a personal history of >10 cumulative colorectal adenomas, a family history of one of the adenomatous polyposis syndromes, or a history of adenomas and FAP-type extracolonic manifestations (duodenal/ampullary adenomas, desmoid tumors (abdominal>peripheral), papillary thyroid cancer, congenital hypertrophy of the retinal pigment epithelium ((CHRPE), epidermal cysts, osteomas) should undergo assessment for the adenomatous polyposis syndromes.
Genetic testing of patients with suspected adenomatous polyposis syndromes should include *APC* and *MUTYH* gene mutation analysis.
Hamartomatous polyposis syndromes
Peutz–Jeghers syndrome (PJS)
Individuals with perioral or buccal pigmentation and/or two or more histologically characteristic gastrointestinal hamartomatous polyp(s) or a family history of PJS should be evaluated for PJS.
Genetic evaluation of a patient with possible PJS should include testing for *STK11* mutations.
Juvenile polyposis syndrome (JPS)
Individuals with five or more juvenile polyps in the colorectum or any juvenile polyps in other parts of the GI tract should undergo evaluation for JPS.
Genetic evaluation of a patient with possible JPS should include testing for *SMAD4* and *BMPR1A* mutations.
Cowden syndrome (PTEN hamartoma tumor syndrome)
Individuals with multiple gastrointestinal hamartomas or ganglioneuromas should be evaluated for Cowden syndrome and related conditions.
Genetic evaluation of a patient with possible Cowden syndrome should include testing for *PTEN* mutations.
Serrated/hyperplastic polyposis syndrome
Individuals who meet at least one of the following criteria have the clinical diagnosis of serrated polyposis syndrome (SPS): (i) at least 5 serrated polyps proximal to the sigmoid colon with ≥2 of these being >10 mm; (ii) any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative (FDR) with serrated polyposis; and (iii) >20 serrated polyps of any size, distributed throughout the large intestine.
A clear genetic etiology has not yet been defined for SPS, and therefore genetic testing is currently not routinely recommended for SPS patients; testing for *MUTYH* mutations may be considered for SPS patients with concurrent adenomas and/or a family history of adenomas.

Tasks to classify incident case

clinical/formal diagnosis

1. familial and personal history
2. phenotype (polyposis, yes or no)
3. young ($\leq$ 50 yrs) age at diagnosis

4. genetic testing

molecular diagnosis

CRC risk in relatives ↑ if juvenile cases occurred within the family

Selected Familial Relative Risks (FRRs) for Probands With Affected First-Degree Relatives (FDRs) Diagnosed at Certain Ages

Proband	No. of probands	FRR (95% CI)
≥1 affected FDR diagnosed <50 y of age	6291	3.31 (2.79–3.89)
≥1 affected FDR diagnosed between 50 and 59 y of age	12,094	2.53 (2.24–2.85)
≥1 affected FDR diagnosed ≥50 y of age	89,340	2.02 (1.93–2.11)

Taylor, *Gastroenterology*, 2010

Predisposition = ↑ risk of CRC

The risk of developing CRC increases in association with specific features **=** excess odds of tumor/cancer development within the same individual/family

- multiple polyps / “polyposis”
- juvenile (age<50 yrs) CRC
- synchronous CRCs
- metachronous CRCs

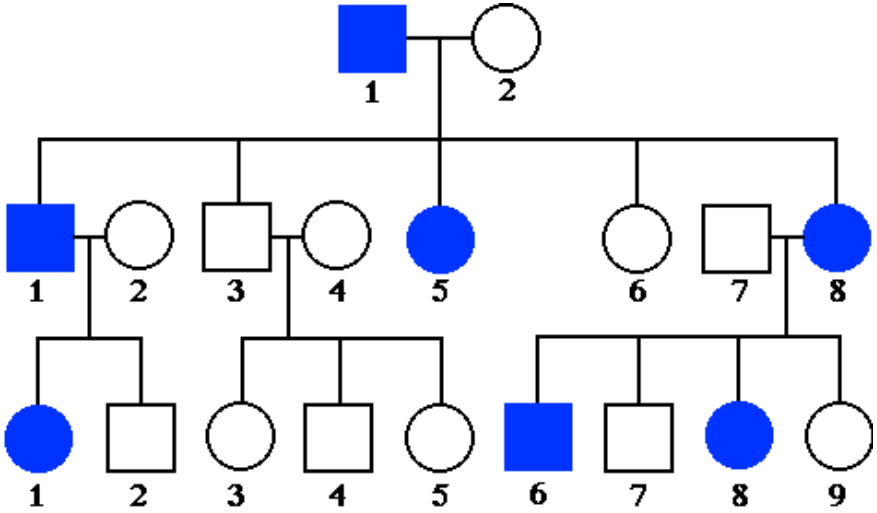
Inherited predispositions to CRC
Phenotypic features and gene defects

Phenotype	Gene defect
• Familial polyposis (FAP)	• ***APC >>MUTYH***
• Attenuated FAP (aFAP)	• ***APC≈MUTYH*** *more data required*
HNPCC	
• Lynch syndrome	• Mismatch Repair Genes ***MLH1≈ MSH2 > MSH6 > PMS2***
• Non Lynch syndr. Familial CRC, or "Familial CRC type X"	**? –** see **NGS**

Most CRC predisposing defects are in dominant genes

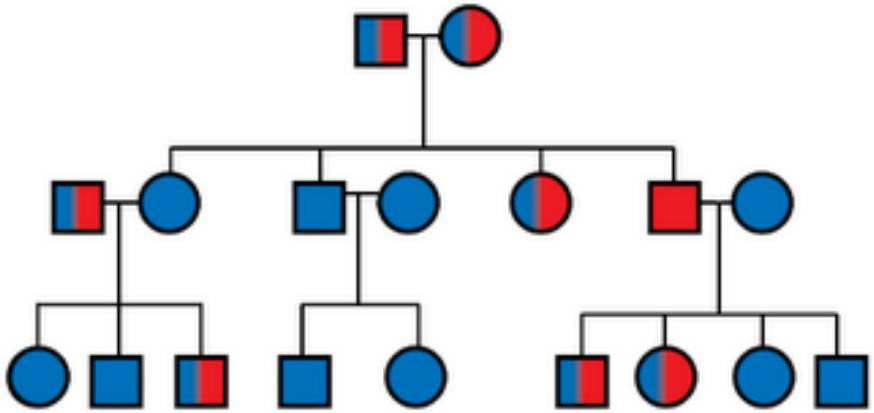
Dominant

APC, Mismatch Repair Genes



Recessive

MUTYH



Hereditary GI Cancer

- The main playground: CRC
  - **Polyposis in all its variants**
  - Lynch syndrome
  - X-syndrome
  - the unknown & the poorly explored

- Other territories
  - Pancreatic cancer
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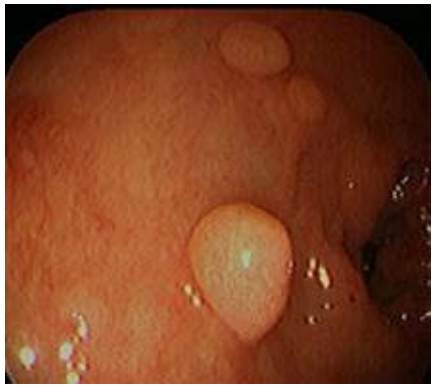
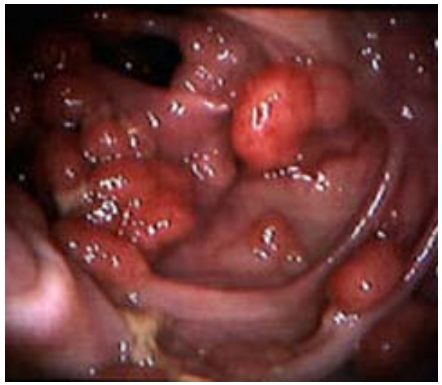
Phenotype: polyps and “polyposis”

The term polyposis should be properly employed.

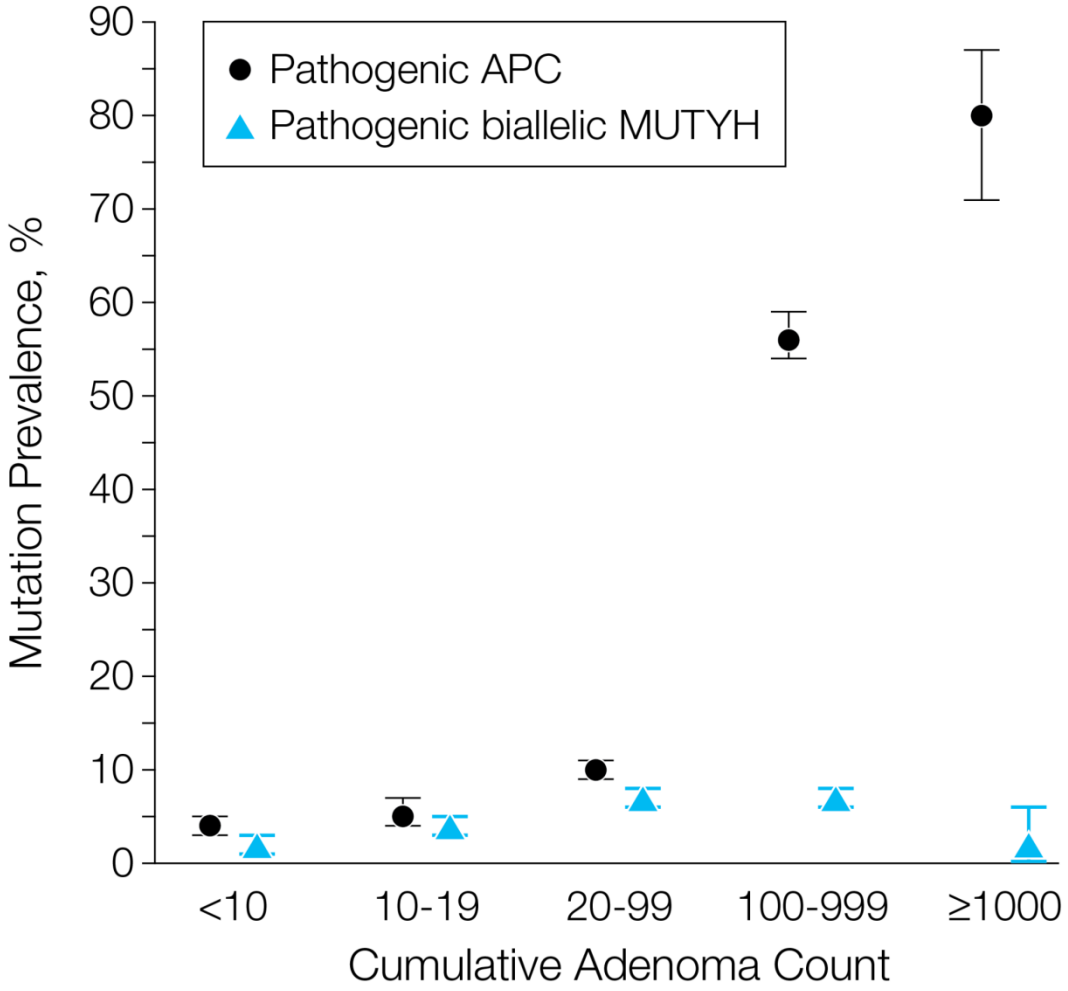
Thresholds



Polyposis, polyp number	>10
Classic/familial polyposis	>100
Attenuated polyposis	<100



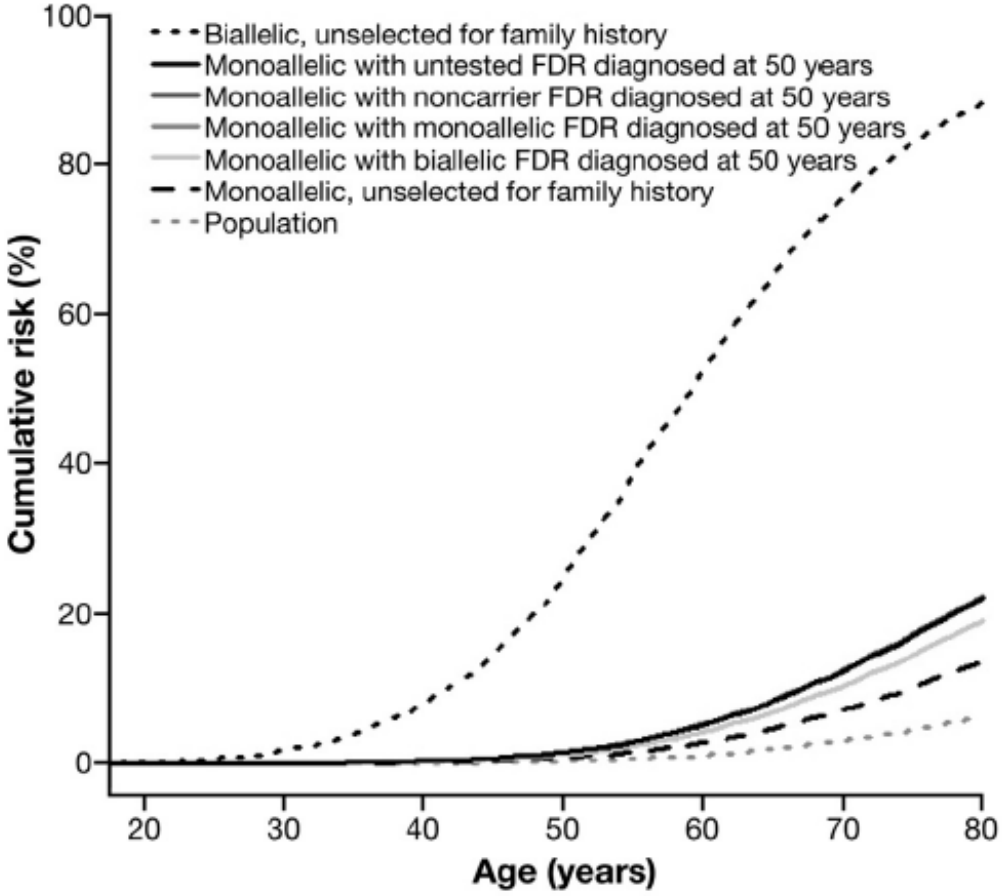
Prevalence of *APC* and *MUTYH* Mutations in Patients with FAP and aFAP



Polyp No	Patient No
>1000	119
100-999	1338
20- 99	3253
10- 19	970

Grover, *JAMA*, 2012

Risk of Colorectal Cancer for Carriers of Mutations in *MUTYH*, With and Without a Family History of Cancer



Study population: 2332 individuals with monoallelic MUTYH mutations among 9504 relatives of 264 CRC cases with MUTYH mutation.

CRC risk through 70 yrs of age:

- males 7.2% (95%CI, 4.6%-11.3%)
- females 5.6% (95%CI, 3.6%- 8.8%)

Gastroenterology 2014

Hereditary GI Cancer

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The University - graduation

Predisposition = ↑ risk of CRC

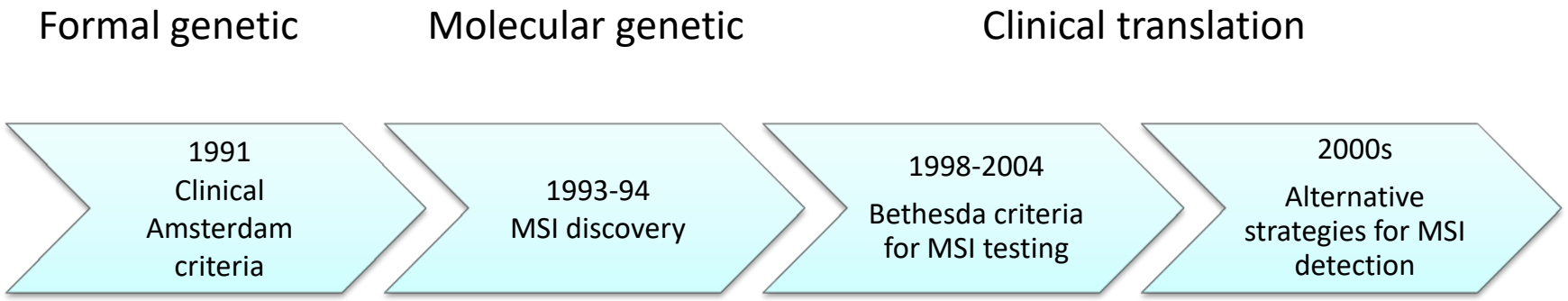
The risk of developing CRC increases in association with specific phenotypic features **=** excess odds of tumor/cancer development within the same individual/family

- multiple polyps / “polyposis”
- juvenile (age<50 yrs) CRC
- synchronous CRCs
- metachronous CRCs

Lynch syndrome

- ≈ 3% of CRC; frequently synchr/metachronus CRC +/- other organs
- autosomal dominant
- CRC risk 60%-80%
- age of onset: medians ranging 44 – 54 yrs
- molecular phenotype=MSI

Lynch syndrome & MSI: from esoterica to standard of care



Keywords

- **Mismatch repair (MMR) defects**
  - lack of function / expression of one of the genes composing the system, i.e. *MLH1*, *MSH2-EPCAM* , *MSH6*, *PMS2*

- **Microsatellite instability (MSI)**
  - hallmark of DNA damage due to defective MMR system

- **Hereditary Non-Polyposis Colorectal Cancer** (**HNPCC)**
  - autosomal dominant inherited predisposition to gastrointestinal/gynecological/other cancers occurring without polyposis

- **Lynch syndrome**
  - autosomal dominant inherited predisposition to gastrointestinal/gynecological/other cancers, due to defective DNA MMR system

Identification of Lynch syndrome
Available tools

- Clinical
  - patient & family hystory=Amsterdam & Bethesda criteria
- Computer models
  - assessing the probability of carrying GL mutations
- Tissue testing
  - Microsatellite instability
  - Immunohistochemistry

Clinical criteria for HNPCC diagnosis

(1991) *Amsterdam I criteria*

Three or more relatives with colorectal cancer plus all of the following:

One affected patient should be a first-degree relative of the other 2;

2 or more successive generations should be affected;

cancer in one or more affected relatives should be diagnosed before the age of 50 years;

familial adenomatous polyposis should be excluded in any cases of colorectal cancer; and

tumors should be verified by pathologic examination.

(1999) *Amsterdam II criteria*

Same as Amsterdam I, except both colon and other HNPCC cancers (endometrial, small bowel, ureteral, or renal pelvis)* can be included to meet the definition.

*Plus: gastric and ovarian cancer

Burt, *Gastroenterology* 2005

Testing CRC for MSI/MMR protein expression: the revised Bethesda Guidelines

Tumors from individuals should be tested for MSI in the following situations:

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age.
2. Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors,* regardless of age.
3. Colorectal cancer with the MSI-H† histology‡ diagnosed in a patient who is less than 60 years of age.§
4. Colorectal cancer or HNPCC-associated tumor* diagnosed under age 50 years in at least one first-degree relative.‖
5. Colorectal cancer or HNPCC-associated tumor* diagnosed at any age in two first- or second-degree relatives.‖

*Endometrial, smoll bowel, urotelial, gastric, and ovarian cancer

† Medullary or Crohn like reaction

Umar, *JNCI* 2004

Molecular tools for the diagnosis of Lynch syndrome

In tumor tissue

- test for MSI, and/or
- MMR defect (immunohistochemistry)
- exclusion of sporadic features (*hMLH1* methylation/*BRAF* mutation)

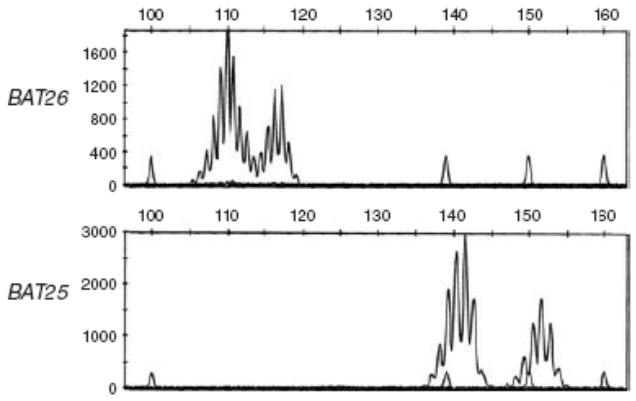
In germ-line

- look for disease-causing mutations in the defective MMR gene: ***MLH1≈ MSH2/EPCAM > MSH6 > PMS2***

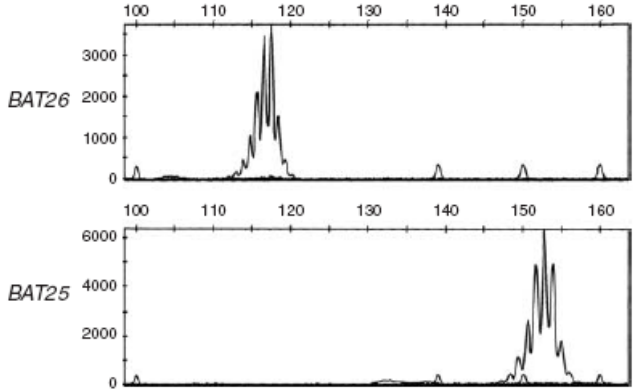
MS-status assessment

PCR of mononucleotide on tumor tissue and analysis of repeat sizes by capillary electrophoresis

MSI



MSS



Mismatch Repair protein loss by immunohistochemistry

Loss of MLH1, MSH2, PMS2, MSH6

Evaluation of germline mutation by sequencing analysis or Multiplex Ligation-dependent Probe Amplification (MLPA) related to HNPCC phenotype

Loss of MLH1

Sporadic phenotype by *BRAF*$^{c.1799\ T>A}$ mutation and absence of germ-line mutations

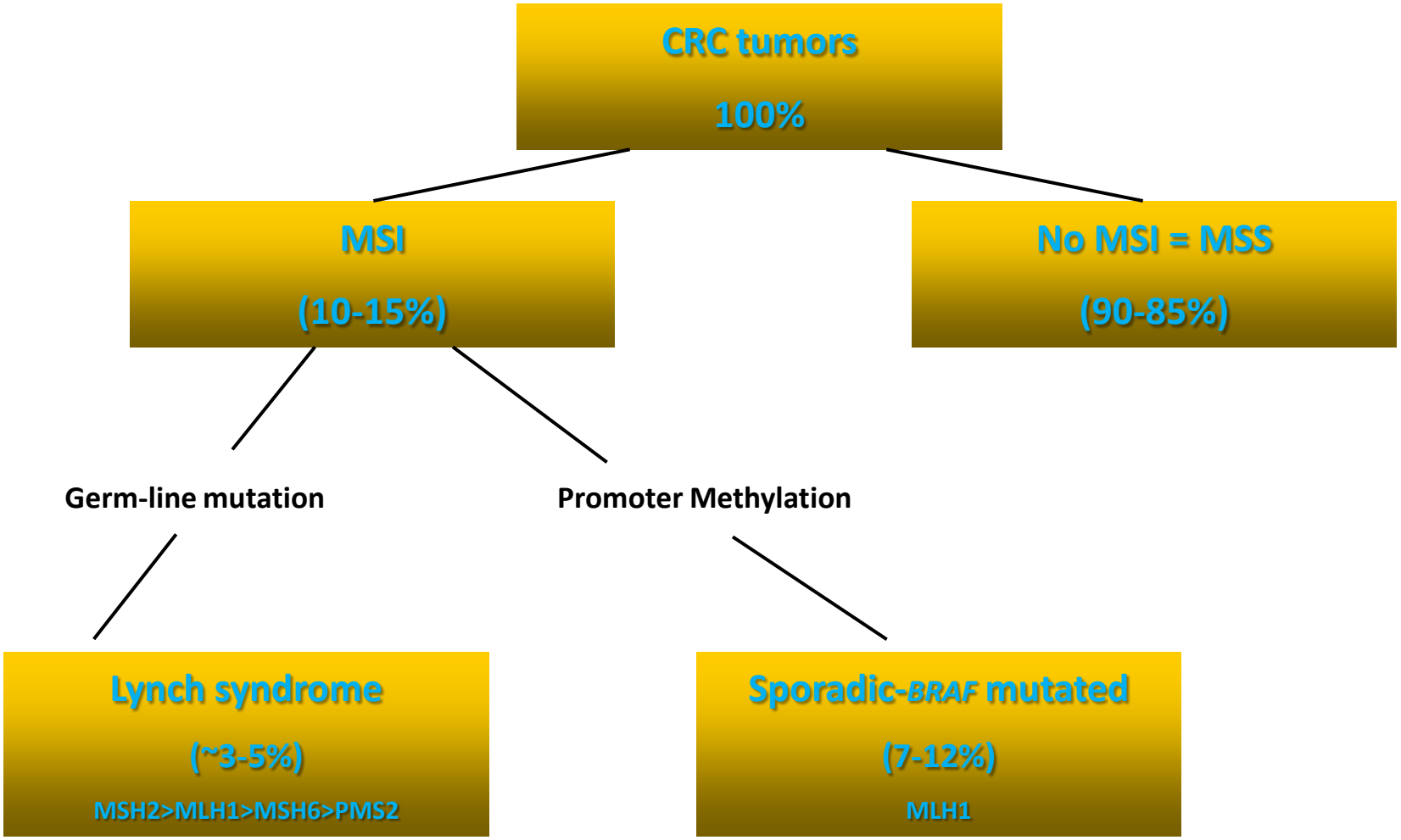
Laghi, Oncogene, 2008

Performance of different strategies for the identification of hMSH2/hMLH1 mutations

Strategy	Sensitivity	Specificity
Fulfillment of revised Bethesda guidelines	90.9	77.1
Presence of MSI	90.9	93.9
Loss of protein expression	81.8	94.2

Pinol, *JAMA*, 2005

Prevalence of MSI CRC



From *De La Chapelle, JCO 2010*

The seminal input: feasibility

The New England Journal of Medicine

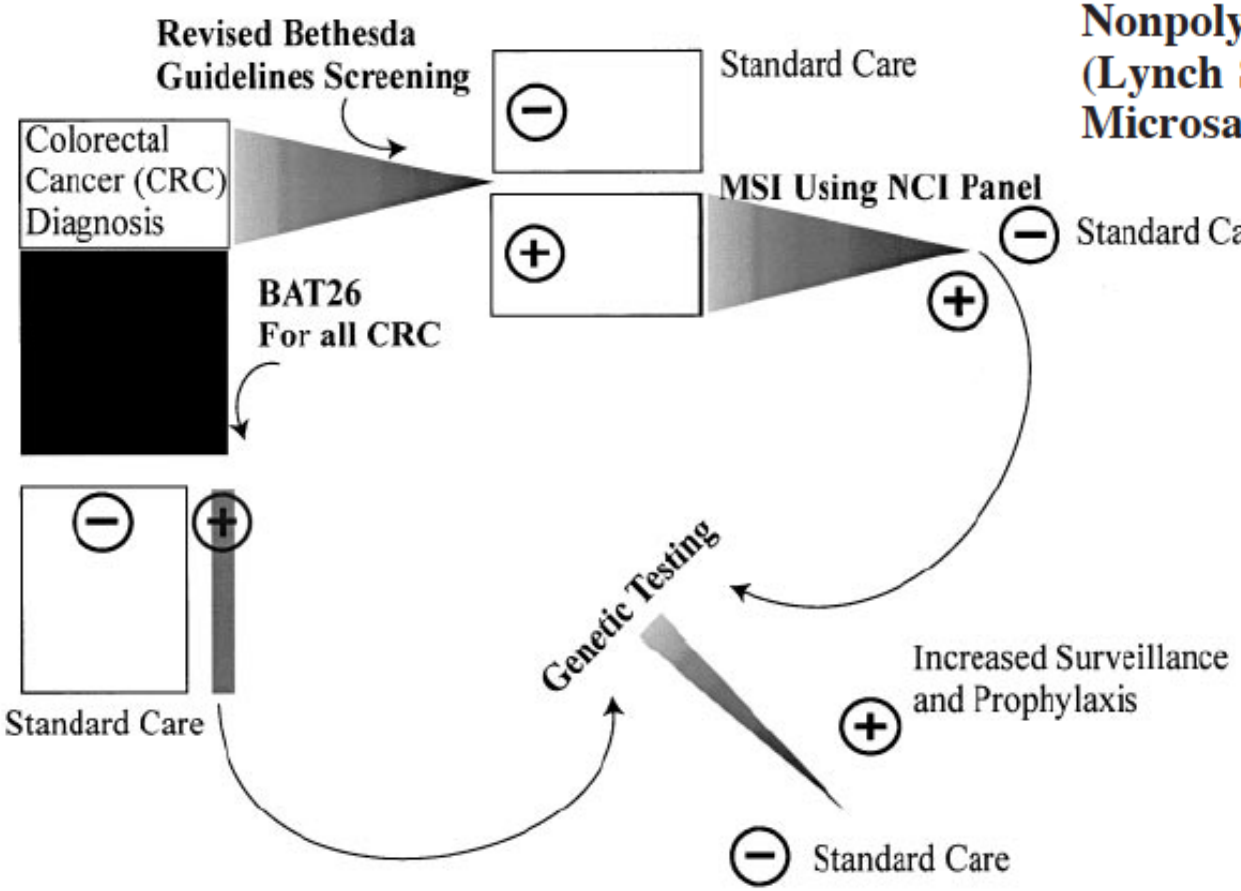
VOLUME 338 MAY 21, 1998 NUMBER 21

INCIDENCE OF HEREDITARY NONPOLYPOSIS COLORECTAL CANCER AND THE FEASIBILITY OF MOLECULAR SCREENING FOR THE DISEASE

LAURI A. AALTONEN, M.D., REIJO SALOVAARA, M.D., PAULA KRISTO, PH.D., FEDERICO CANZIAN, PH.D., AKSELI HEMMINKI, M.B., PÄIVI PELTOMÄKI, M.D., ROBERT B. CHADWICK, M.SC., HELENA KÄÄRIÄINEN, M.D., MATTI ESKELINEN, M.D., HEIKKI JÄRVINEN, M.D., JUKKA-PEKKA MECKLIN, M.D., AND ALBERT DE LA CHAPELLE, M.D.

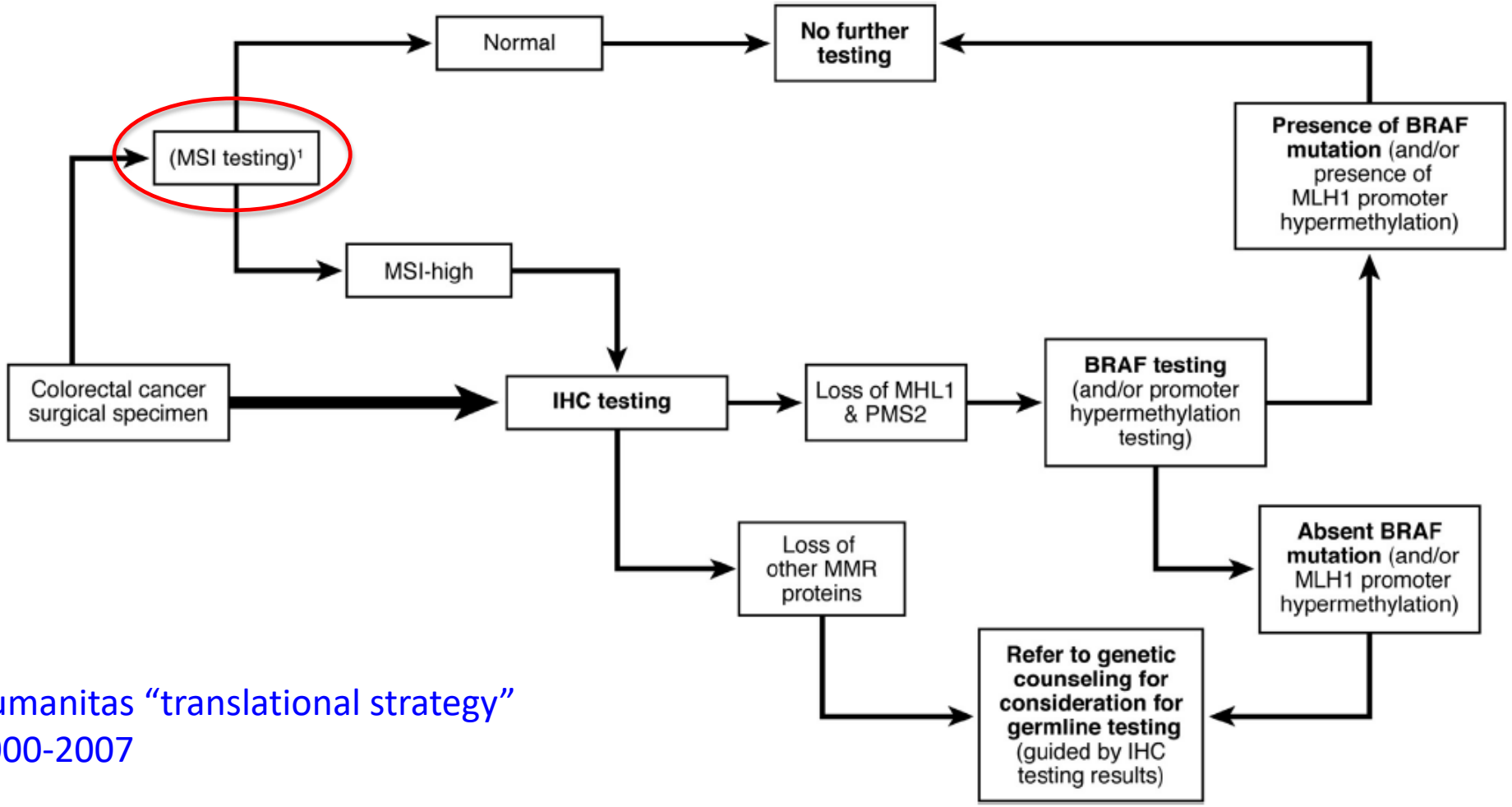
Guidelines *vs* universal screening: the debate

Re: Revised Bethesda Guidelines for Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndrome) and Microsatellite Instability



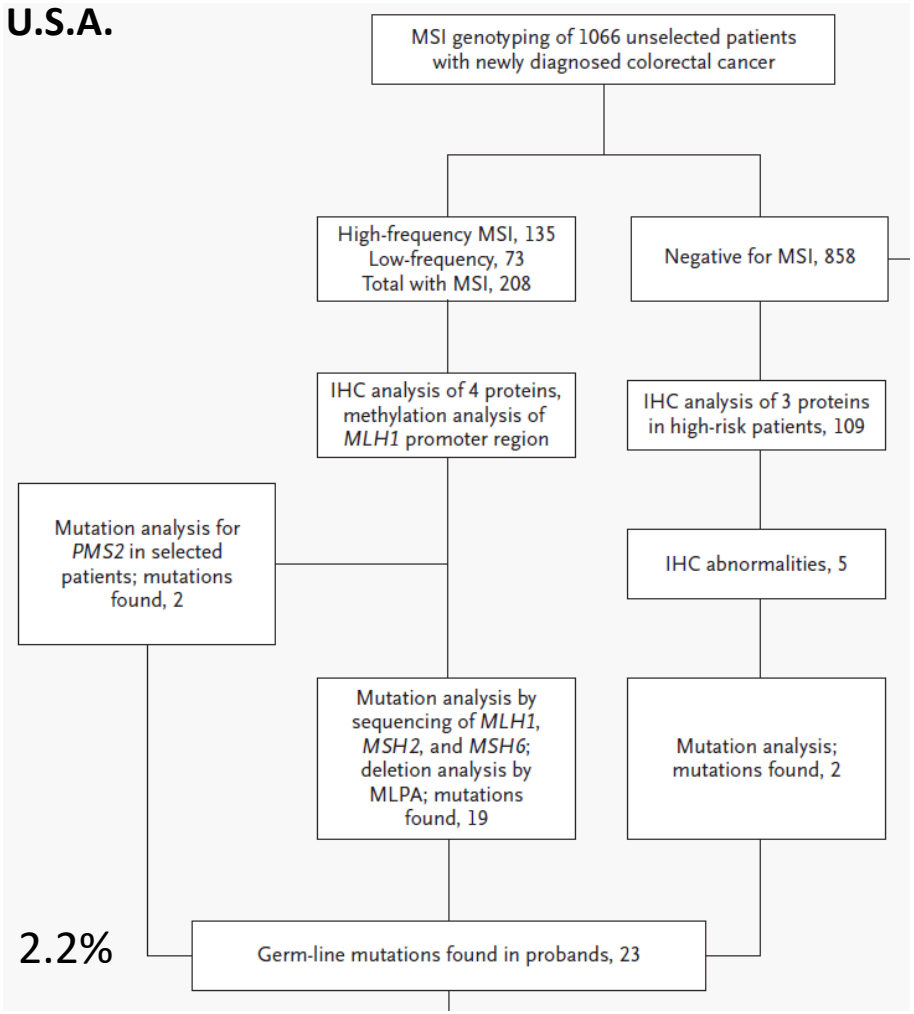
Journal of the National Cancer Institute, Vol. 96, No. 18, September 15, 2004

Identification of Lynch syndrome: Universal screening by tumor testing

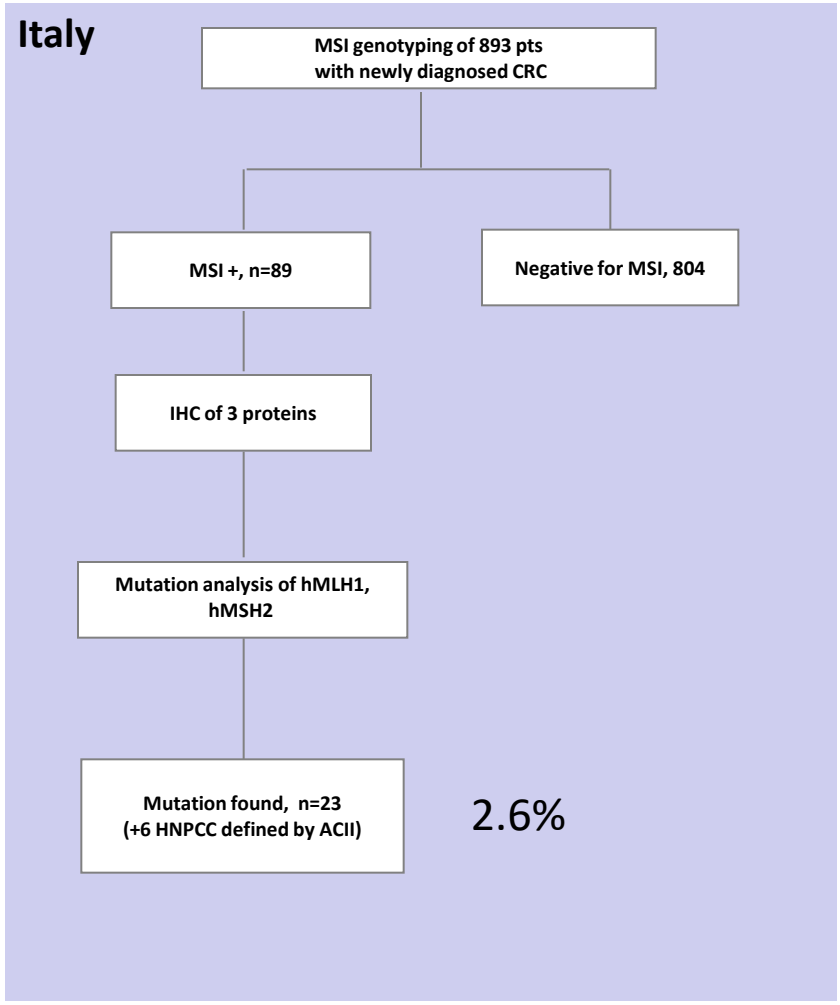


Humanitas "translational strategy"
2000-2007

MSI screening for Lynch diagnosis



Hampel, NEJM, 2005



Malesci, Clin Cancer Res, 2007

Guidelines on Genetic Evaluation and Management of Lynch Syndrome: A Consensus Statement by the US Multi-Society Task Force on Colorectal Cancer

The Multi-Society Task Force, in collaboration with invited experts, developed guidelines to assist health care providers with the appropriate provision of genetic testing and management of patients at risk for and affected with Lynch syndrome

This article is being published jointly in *Gastroenterology, American Journal of Gastroenterology, Diseases of the Colon & Rectum, and Gastrointestinal Endoscopy.*

PRACTICAL GUIDELINES *July 2014*

Familial risk-colorectal cancer: ESMO Clinical Practice Guidelines†

Annals of Oncology

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

JSMO Japanese Society of Medical Oncology

VOLUME 33 · NUMBER 2 · JANUARY 10 2015

JOURNAL OF CLINICAL ONCOLOGY

ASCO SPECIAL ARTICLE

Hereditary Colorectal Cancer Syndromes: American Society of Clinical Oncology Clinical Practice Guideline Endorsement of the Familial Risk–Colorectal Cancer: European Society for Medical Oncology Clinical Practice Guidelines

- Tumor testing ***for DNA mismatch repair (MMR) deficiency*** with immunohistochemistry for MMR proteins and/or MS should be ***assessed*** in all CRC patients. As an alternate strategy, tumor testing should be carried out in individuals with CRC younger than 70 years, or those older than 70 years who fulfill any of the revised Bethesda guidelines (Table 1).

Interval cancers* and MSI

		Interval cancer (n=46)	Non- Interval cancer (n=97)
MSI +		14 (30)	10 (10)
MSS		32 (70)	87 (90)

P=0.003

MSI phenotype for interval cancers, O.R. 3.7, 95%C.I., 1.5-9.1

*51/993 cancers, defined as CRC that developed within 5 years of a complete clean colonoscopy

Sawhney, *Gastroenterology*, 2006

Very high yield of MSI/Lynch syndrome in selected subsets

- CRC patients with
  - Juvenile onset
  - Synchronous tumors
  - Metachronous tumours

have a frequency of MSI cancers >>10%

or of patients with Lynch syndrome >> 3%

MMR story: lessons from a long-standing biomarker

First exa … medicine

- late 90s', b … d tumor predisposition
- early 2000s
  - differen … survival
  - … stro …
- 2010s'…, n … (… OX) for TSN … 0 colorectal cancers (n …)
- >2015s', d … ess (stage IIIB)?
- nowadays: … py responsive …
- what's next

Hereditary GI Cancer

- The main playground: CRC
  - Polyposis in all its variants
  - Lynch syndrome
  - **X-syndrome**
  - the unknown & the poorly explored

- Other territories
  - Pancreatic cancer
  - Gastric cancer

Familial Colorectal Cancer – Type X

Family history of CRC fulfilling AC but without MSI/MMR defects

Table 1. Standardized Incidence Ratios Comparing First- and Second-Degree Relatives of Group A vs Group B

	Group A (MSI-H) (n = 1855)*		Group B (MSI-L/MSS) (n = 1567)*		
Tumor Site	No. of Tumors (Men/Women)	SIR (95% CI)†	No. of Tumors (Men/Women)	SIR (95% CI)†	*P* Value‡
Colorectum	182 (94/88)	6.1 (5.2-7.2)§	55 (23/32)	2.3 (1.7-3.0)§	<.001
Uterus	41 (0/41)	4.1 (2.9-5.6)§	6 (0/6)	0.8 (0.3-1.6)	<.001
Stomach	21 (14/7)	4.6 (2.7-6.6)§	5 (1/4)	1.4 (0.3-2.8)	.008

Lindor, *JAMA*, 2005

Familial Colorectal Cancer – Type X

- Nearly half of 161 familial clusters are not Lynch Syndrome.
  - ≈ 40% of tumors do not have MSI or abnormal IHC for DNA MMR proteins
  - Lower penetrance for CRC; later onset of CRC
  - SIR for CRC ≈ 2.3
  - No excess of non colorectal cancers

(Colon Cancer Family Registry, Lindor et al. JAMA 293:1979, 2005)

- Multiple genes are being reported for this, but none are common to multiple families (*GalNT12,BMPR1A, RPS20, SEMA4A, HNRPA0, WIF1*)

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  - X-syndrome
  - **the unknown & the poorly explored**

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Innovation - unknown

Science

Few men story…

Remains true and holds over time

Technology

Few men + lot of money …

Usually abandoned over time

GASTROENTEROLOGY 2013;144:1402–1409

Prevalence of Germline *PTEN, BMPR1A, SMAD4, STK11,* and *ENG* Mutations in Patients With Moderate-Load Colorectal Polyps

Table 2. Patient Demographics (N = 603)

Clinical characteristics	N = 603
Age at presentation of 5th polyp, *y*, median (range)	51 (2–89)
Sex, n (%)	
Female	360 (59.7)
Male	243 (40.3)
No. of polyps, median (range)	13 (5–302)
No. of scopes, median (range)	3 (1–19)
Personal history of CRC, n (%)	119 (19.7)
Age of onset of CRC, *y*, median (range)	53 (21–80)
Family history of CRC, n (%)	
Any in 3-generation pedigree	325 (53.8)
First-degree relative	186 (30.8)
Family history of polyps	295 (48.9)
Clinical criteria met, n (%)	
JPS	69 (11.4)
PJS	20 (3.3)
MAP	39 (6.5)
HNPCC	10 (1.7)
FAP	2 (0.3)
AFAP	43 (7.1)
SPS	45 (7.5)
None	440 (73.0)

AFAP, attenuated familial adenomatous polyposis; FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer; JPS, juvenile polyposis syndrome; MAP, *MUTYH*-associated polyposis; PJS, Peutz-Jeghers syndrome; SPS, serrated polyposis syndrome.

Table 3. Univariate Risk Factors (Clinical Characteristics) for Germline Mutations in *ENG, PTEN, STK11, BMPR1A,* and *SMAD4*

		ENG (n = 11 [1.8%])		*PTEN* (n = 13 [2.2%])		*STK11* (n = 13 [2.2%])		*BMPR1A* (n = 20; [3.3%])		*SMAD4* (n = 21 [3.5%])		Any gene (n = 77 [12.8%])	
Variable	N	n	%	n	%	n	%	n	%	n	%	n	%
Clinical characteristics													
Age, *y*													
<40	155	3	1.9	3	1.9	7	4.5	6	3.9	11	7.1	30	19.4
≥40	448	8	1.8	10	2.2	6	1.3	14	3.1	10	2.2	47	10.5
P value		1.0		1.0		**.047**		.61		**.009**		**.008**	
Sex													
Female	360	5	1.4	7	1.9	4	1.1	11	3.1	10	2.8	37	10.3
Male	243	6	2.5	6	2.5	9	3.7	9	3.7	11	4.5	40	16.5
P value		.36		.78		**.044**		.65		.26		**.034**	
No. of polyps													
5–29	461	10	2.2	8	1.7	9	2.0	9	2.0	14	3.0	50	10.8
≥30	142	1	0.7	5	3.5	4	2.8	11	7.7	7	4.9	27	19.0
P value		.47		.20		.52		**.002**		.30		**.014**	
Family history of colonic polyps													
No	308	5	1.6	8	2.6	7	2.3	7	2.3	5	1.6	32	10.4
Yes	295	6	2.0	5	1.7	6	2.0	13	4.4	16	5.4	45	15.3
P value		.77		.58		1.0		.17		**.013**		.09	
Personal history of CRC													
No	484	7	1.4	11	2.3	12	2.5	16	3.3	20	4.1	65	13.4
Yes	119	4	3.4	2	1.7	1	0.8	4	3.4	1	0.8	12	10.1
P value		.24		1.0		.48		1.0		.10		.36	
Family history of CRC													
No	278	5	1.8	13	4.7	8	2.9	10	3.6	16	5.8	51	18.3
Yes	325	6	1.8	0	0.0	5	1.5	10	3.1	5	1.5	26	8.0
P value		1.0		**<.001**		.28		.82		**.006**		**<.001**	

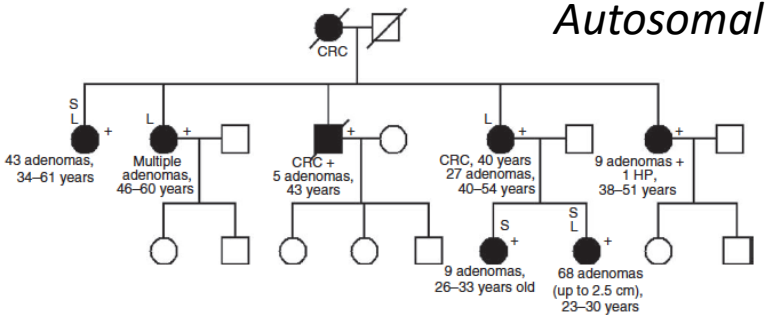
NOTE. Significant *P* values (<.05) are shown in bold type.

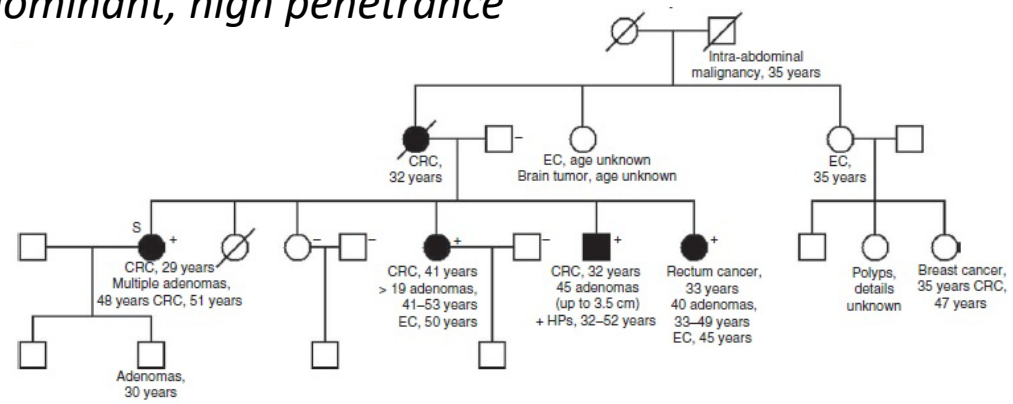
Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas

Selected aFAP families (APC & MUYH negative)

- **POLEp.Leu424Val** multiple polyps/CRC, early onset
- **POLD1p.Ser478Asn** multiple polyps/CRC, early onset + endometrial cancer

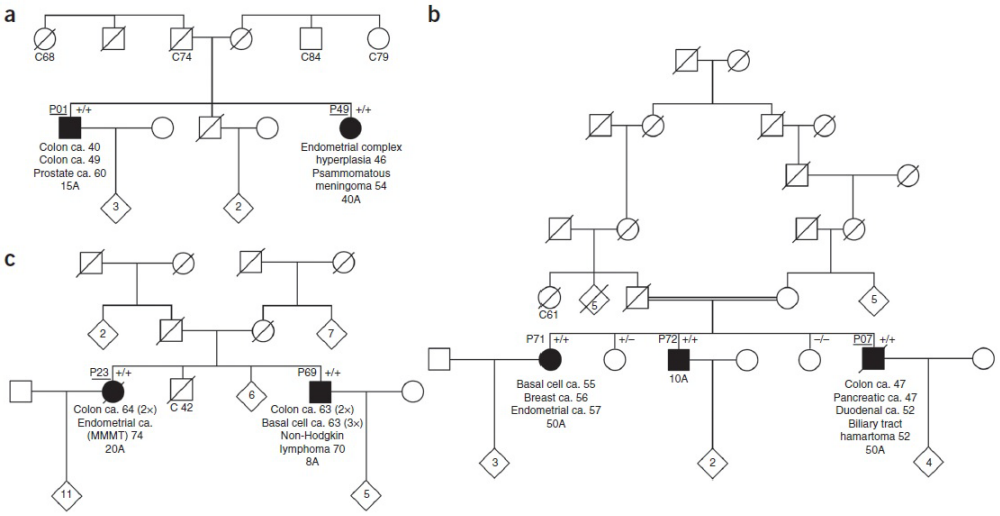
Autosomal dominant, high penetrance





VOLUME 47 | NUMBER 6 | JUNE 2015 NATURE GENETICS

A germline homozygous mutation in the base-excision repair gene *NTHL1* causes adenomatous polyposis and colorectal cancer



Autosomal recessive inheritance

WES of 51 individuals from 48 families:
7 individuals from 3 unrelated families harbored homozygous mutations
+endometrial malignancy in women

Table 1 Nonsense germline mutations in BER pathway genes

Family	Subject	Gene	Variant[a]	Protein alteration	Allelic state	Clinical features of the patient[b]
A	P01	*NTHL1*	c.268C>T	p.Gln90*	Homozygous	C40, C49, 15A
A	P49	*NTHL1*	c.268C>T	p.Gln90*	Homozygous	40A
B	P07	*NTHL1*	c.268C>T	p.Gln90*	Homozygous	C47, 50A
B	P71	*NTHL1*	c.268C>T	p.Gln90*	Homozygous	50A
B	P72	*NTHL1*	c.268C>T	p.Gln90*	Homozygous	10A
C	P23	*NTHL1*	c.268C>T	p.Gln90*	Homozygous	C64 (2×), 20A
C	P69	*NTHL1*	c.268C>T	p.Gln90*	Homozygous	C63 (2×), 8A
D	P54	*OGG1*	c.391C>T	p.Arg131*	Heterozygous	13A + 8H
E	P57	*MPG*	c.352C>T	p.Arg118*	Heterozygous	15A
F	P09	*SMUG1*	c.370C>T	p.Arg124*	Heterozygous	C49, 20A

[a]All variants were validated by Sanger sequencing. [b]C, colorectal cancer; A, adenomatous polyps; H, hyperplastic polyps. Numbers represent age (in years) of onset of CRC (C) or the number of adenomatous (A) or hyperplastic (H) polyps present at the time of diagnosis.

nature genetics

Synopsis of inherited polyposis

Syndromes		History					Inheritance	
		Burden and age at onset			Additional features	Family	Pattern	Gene
		Number	Polyps (yrs)	CRC (yrs)				
Adenomatous	Familial Adenomatous Polyposis (FAP)	>20 to thousands	16	39	Gardner, Turcot[a]	Positive	Dominant	***APC***
	MYH-associated Polyposis (MAP)	>10 to hundreds[b]	50	45–59		Negative	Recessive	***MUTYH***
	Polymerase Proofreading-associated Polyposis (PPAP)[c]	20–100	16–74	26–78		Positive	Dominant	***POLE, POLD1***
	NTHL1- associated Polyposis	8–50	≈50	40–67		Negative	Recessive	***NTHL1***
	MSH3-associated polyposis	<100	30–50	Late onset		Negative	Recessive	***MSH3***
Non-Adenomatous	Peutz-Jeghers	Multiple[d]	10-…	43		Positive	Dominant	***STK11***
	Hamartoma Tumor	Multiple[d]	10–15	38–46	Cowden[e], BRRS[f]	Positive	Dominant	***PTEN***
	Juvenile Polyposis	4 to >100[d,g]	20≤	34		Positive	Dominant	***SMAD4, BMPR1A***
	Hereditary Mixed Polyposis	Multiple	40-50?	?		Positive	Dominant	***GREM1***
	Serrated Polyposis Syndrome[h,i]	5–20[9]	30–40	?		Positive	Dominant	***RNF43***

The puzzle of inherited polyposis

Complex Disease Scenario

Multifactorial Puzzle

Inheritance Pattern & Gene Mutation

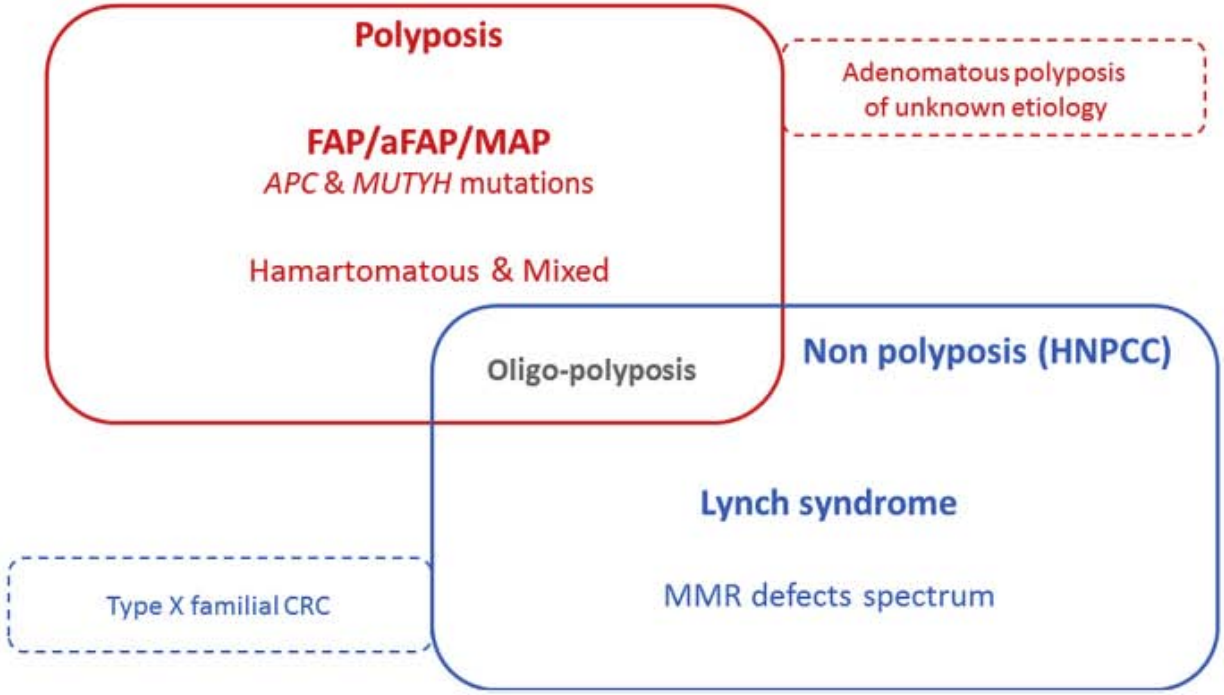
Individual features

Familial & Personal History

Polyp number & Histology

G. Basso et al. / Best Practice & Research Clinical Gastroenterology 31 (2017)

Simplified nosography of CRC predispositions



G. Basso et al. / Best Practice & Research Clinical Gastroenterology 31 (2017)

JAMA Oncol. doi:10.1001/jamaoncol.2015.0197
Published online March 12, 2015.

Hypothesis driven

Original Investigation

Germline *TP53* Mutations in Patients With Early-Onset Colorectal Cancer in the Colon Cancer Family Registry

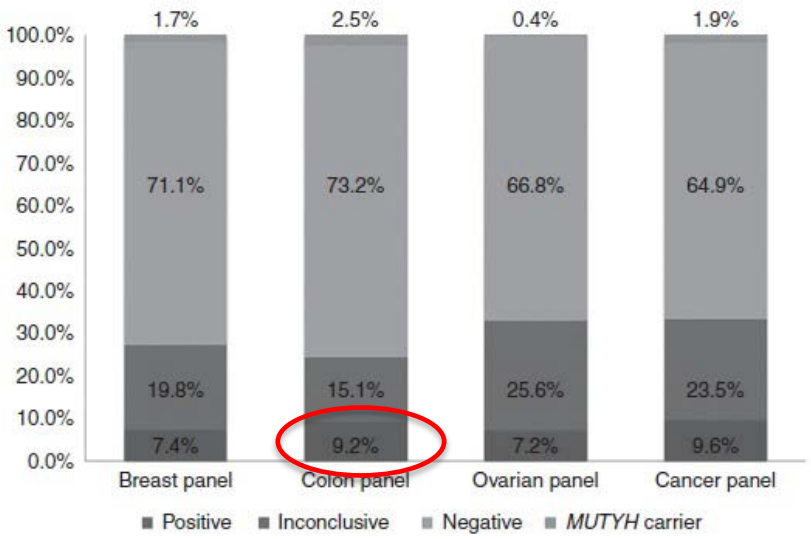
RESULTS Among 457 eligible participants (314, population-based; 143, clinic-based; median age at diagnosis, 36 years [range, 15-40 years]), 6 (1.3%; 95% CI, 0.5%-2.8%) carried germline missense *TP53* alterations, none of whom met clinical criteria for Li-Fraumeni syndrome. Four of the identified *TP53* alterations have been previously described in the literature in probands with clinical features of Li-Fraumeni syndrome, and 2 were novel alterations.

CONCLUSIONS AND RELEVANCE In a large cohort of patients with early-onset colorectal cancer, germline *TP53* mutations were detected at a frequency comparable with the published prevalence of germline *APC* mutations in colorectal cancer. With the increasing use of multigene next-generation sequencing panels in hereditary cancer risk assessment, clinicians will be faced with the challenge of interpreting the biologic and clinical significance of germline *TP53* mutations in families whose phenotypes are atypical for Li-Fraumeni syndrome.

Utilization of multigene panels in hereditary cancer predisposition testing: analysis of more than 2,000 patients

Table 3 Result rates by panel and clinician-reported clinical history

Characteristic (total cases)	Positive, *n* (%)	Inconclusive, *n* (%)	Negative[a], *n*(%)	Mutation-positive genes (no. of mutations/likely pathogenic variants)
Colon panel (557)	51 (9.2)[b]	84 (15.1)	408 (73.2)	*MSH2* (7), *MLH1* (7)[c], *APC* (6), *CHEK2* (6)[c], *MUTYH* biallelic (6), *PMS2* (6), *MSH6* (5)[c], *SMAD4* (4), *PTEN* (3), *CDH1* (1), *STK11* (1), *TP53* (1)
CRC dx <50 years (168)	22 (13.1)[b]	23 (13.7)	120 (71.4)	*MLH1* (6)[c], *MSH2* (3), *MUTYH* biallelic (3), *PMS2* (3), *APC* (2), *CHEK2* (2)[c], *MSH6* (3)[c], *SMAD4* (2)
2–9 Cumulative adenomas (120)	9 (7.5)[c]	25 (20.8)	84 (70.0)	*APC* (2), *CHEK2* (2)[c], *MSH2* (2), *MLH1*(2)[c], *PMS2*(1), *PTEN* (1)
10+ Cumulative adenomas (90)	13 (14.4)	11 (12.2)	63 (70.0)	*MUTYH* (3), *APC* (2), *PTEN* (2), *PMS2* (2), *CDH1* (1), *CHEK2* (1), *MLH1* (1), *SMAD4* (1)



Clinician-referred patients
Results from commercial multi-gene panels assessing 14-22 genes (BRACA1/2 excluded)

2014 Genetics inMedicine

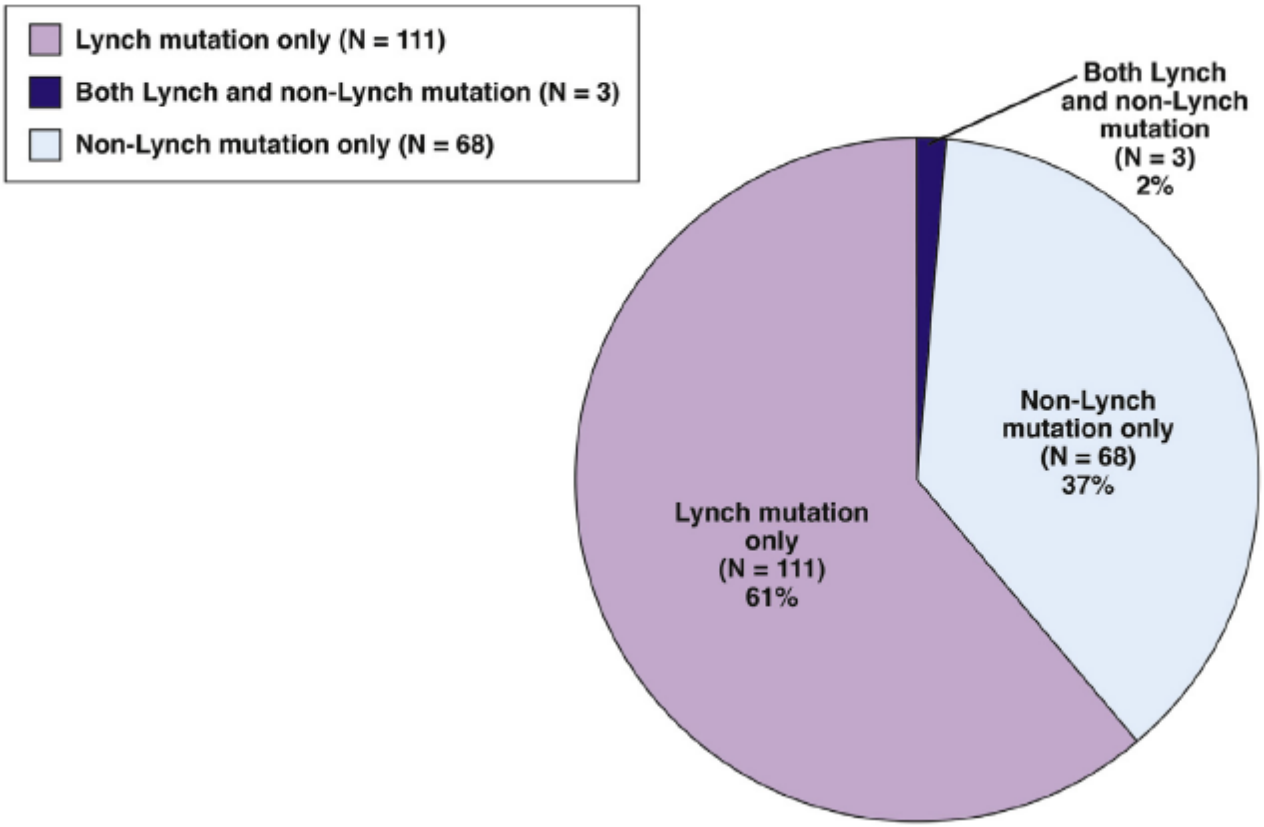
New data by “massive” sequencing

Author	Study type	Setting	Patients	Mutational gain	
				N	Prevalence (95%CI)
Yourgelun M, 2016	Cross sectional	Folowing LS assessment 2012-13*	1260	185	14.4% (12.6%-16.5%)
Yourgelun M, 2017	Cohort	Dana Farber CRCs, 2008-14§	1058	115	9.9% (8.2%-12.9%)
Pearlman R, 2017	Ohio State	Juvenile CRC@	450	72	16.0% (12.8%-19.8%)

*Commercial test provided by Myriad genetics
§ Commercially available, by Myriad genetics
@ MSI/MMR test first, then appropriate testing, by Myriad genetics

Too much commercial?

Identification of a Variety of Mutations in Cancer Predisposition Genes in Patients With Suspected Lynch Syndrome



Yourgelun, *Gastroenterology*, 2016

Identification of a Variety of Mutations in Cancer Predisposition Genes in Patients With Suspected Lynch Syndrome

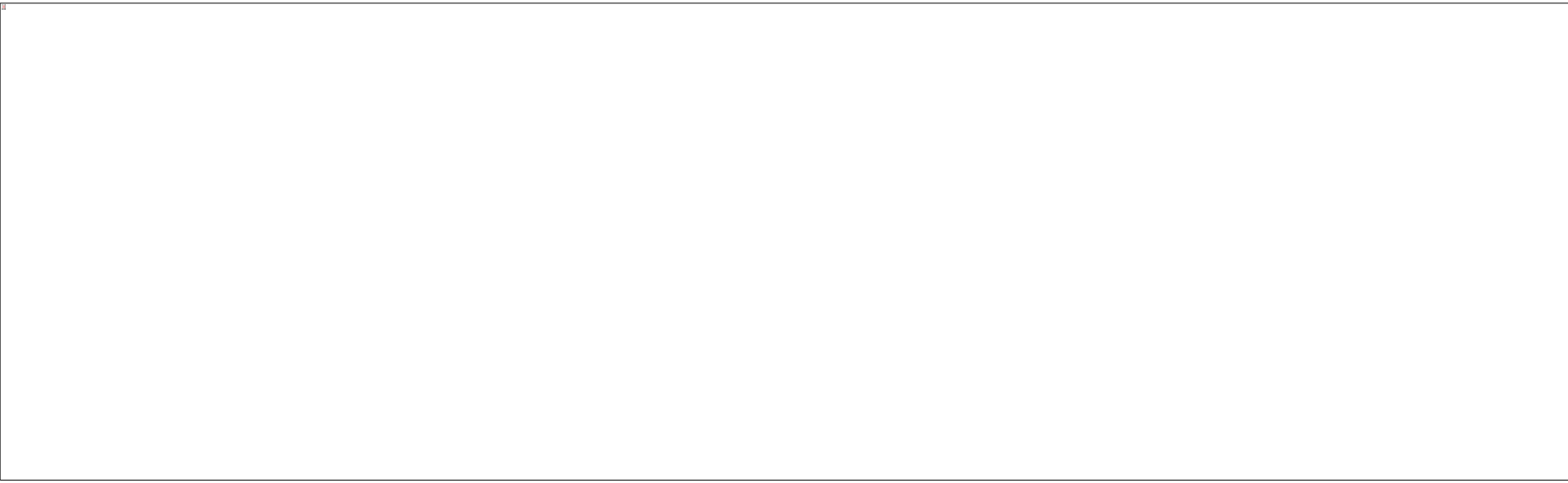
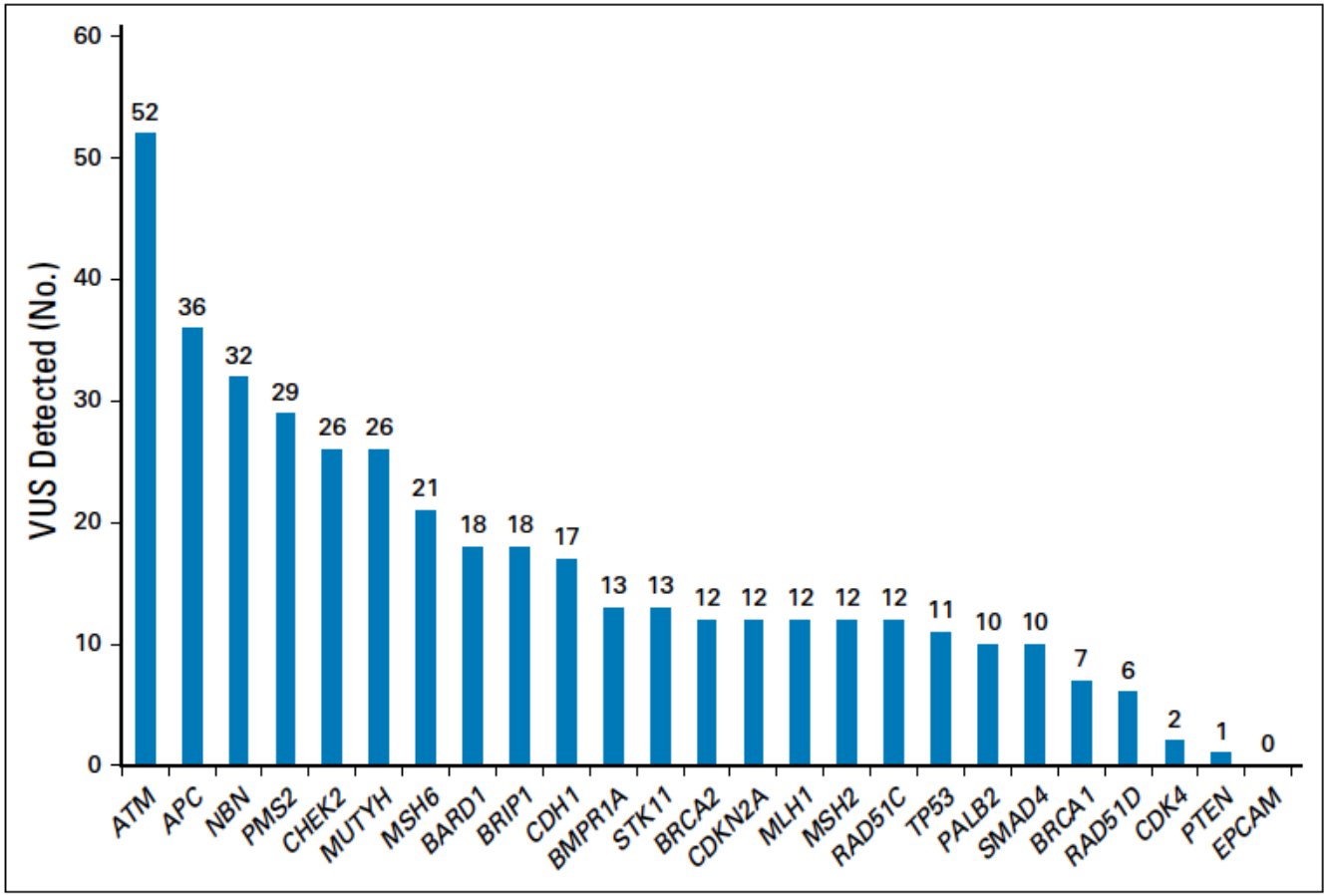


Figure 1. Pathogenic mutations identified with a multigene panel among 1260 individuals with suspected Lynch syndrome. (*A*) Proportion of mutation carriers with Lynch syndrome mutations (purple), non–Lynch syndrome mutations (blue), or both Lynch and non–Lynch syndrome mutations (dark purple). (*B*) Distribution of Lynch syndrome mutation carriers by specific gene. (*C*) Distribution of non–Lynch syndrome mutation carriers by gene type (*BRCA1/2*, monoallelic *MUTYH*, other high-penetrance genes, or moderate-penetrance genes).

Yourgelun, *Gastroenterology*, 2016

The “VUS” issue



Number of germline variants of uncertain significance (VUS), per gene, detected with a 25-gene panel in 1,058 patients with colorectal cancer.

Yourgelun, *JCO*, 2017

Unexpected findings in juvenile CRC by “massive” sequencing

Gene	Associated Syndrome or Cancer(s)	Overall Penetrance	Patients With Mutation, No. (%)	(95% CI)
Any pathogenic or likely pathogenic mutation			72 (16)	(12.8-19.8)
Genes associated with colon cancer			59 (13.1)	(10.2-16.7)
MLH1	Lynch syndrome	High	13 (2.9)	(1.6-5.0)
MSH2	Lynch syndrome	High	16 (3.6)	(2.1-5.8)
MSH2/monoallelic *MUTYH*	Lynch syndrome/colon cancer	High/low	1 (0.2)	(0.01-1.4)
MSH6	Lynch syndrome	Moderate	2 (0.4)	(0.08-1.8)
PMS2	Lynch syndrome	Moderate	5 (1.1)	(0.4-2.7)
APC	Familial adenomatous polyposis (FAP)	High	5 (1.1)	(0.4-2.7)
APC p.I1307K	Colon cancer	Low	4 (0.9)	(0.3-2.4)
MUTYH				
Biallelic	MUTYH-associated polyposis (MAP)	High	4 (0.9)	(0.3-2.4)
Monoallelic	Colon cancer	Low	7 (1.6)	(0.7-3.3)
SMAD4	Juvenile polyposis syndrome	High	1 (0.2)	(0.01-1.4)
APC/PMS2	FAP/Lynch syndrome	High/moderate	1 (0.2)	(0.01-1.4)
Genes not traditionally associated with colon cancer			13 (2.9)	(1.6-5.0)
BRCA1	Hereditary breast-ovarian cancer syndrome	High	2 (0.4)	(0.08-1.8)
BRCA2	Hereditary breast-ovarian cancer syndrome	High	4 (0.9)	(0.3-2.4)
ATM	Breast cancer, pancreatic cancer	Moderate	3 (0.7)	(0.2-2.1)
ATM/CHEK2	Breast cancer, pancreatic cancer	Moderate	1 (0.7)	(0.01-1.4)
PALB2	Breast cancer, pancreatic cancer	Moderate	2 (0.4)	(0.08-1.8)
CDKN2A	Melanoma, pancreatic cancer	High	1 (0.2)	(0.01-1.4)

= 3.3%

> LS prevalence?

Pearlman, *JAMA Oncol*, 2017

Where is genetic testing of CRC tumors in 2017

- Sanger sequencing – traditional method
- Then Next generation sequencing (NGS)
- NGS – decreased cost, increased efficiency
- Usher in era of multiplex genetic testing

But, what the gastroenterologist should do?

- Be suspicious and aware of technology
- Think of large data-bases: contribute even only 1 case
- Be aware of business interference as well of limitations
- Change both the “scope” and the perspective
- Collaborate and individualize patient approach

The precision medicine initiative

The NEW ENGLAND JOURNAL of MEDICINE

A New Initiative on Precision Medicine

Francis S. Collins, M.D., Ph.D., and Harold Varmus, M.D.

Perspective

FEBRUARY 26, 2015

"Tonight, I'm launching a new Precision Medicine Initiative to bring us closer to curing diseases like cancer and diabetes — and to give all of us access to the personalized information we need to keep ourselves and our families healthier."

— President Barack Obama, State of the Union Address, January 20, 2015

"Doctors have always recognized that every patient is unique, and doctors have always tried to tailor their treatments as best they can to individuals. You can match a blood transfusion to a blood type — that was an important discovery. What if matching a cancer cure to our genetic code was just as easy, just as standard? What if figuring out the right dose of medicine was as simple as taking our temperature?"

President Obama, January 20, 2015

U.S. Department of Health & Human Services

NIH National Institutes of Health

Turning Discovery Into Health

NGS: types of panels

- Syndrome specific tests
- Cancer specific high penetrance panel
- Cancer specific high and moderate penetrance
- Comprehensive panels

Academic offer

Individualized Medicine Clinic: Patient Care

MAYO CLINIC
Mayo Medical Laboratories

Test ID: HCCP

Hereditary Colon Cancer Multi-Gene Panel

Gene	Known Association
MLH1	Lynch syndrome
MSH2	Lynch syndrome
MSH6	Lynch syndrome
PMS2	Lynch syndrome
EPCAM	Lynch syndrome
APC	Familial adenomatous polyposis
MYH/MutYH	*MYH*-associated polyposis
SCG5/GREM1	Hereditary mixed polyposis syndrome
STK11	Peutz-Jeghers syndrome
SMAD4	Juvenile polyposis syndrome
PTEN	*PTEN* hamartoma tumor syndrome (ie, Cowden syndrome)
CDH1	Hereditary diffuse gastric cancer
AXIN2	Oligodontia-colorectal cancer syndrome
TP53	Li-Fraumeni syndrome
CHEK2	Low-risk gene
MLH3	Low-risk gene

Company offer

Table Summary of Genes and Associated Cancers

myRisk Hereditary Cancer

BR = Breast
OV = Ovarian
CO = Colorectal
EN = Endometrial
ME = Melanoma
PA = Pancreatic
GA = Gastric
PR = Prostate
OC = Other Cancers / Clinical Features

Gene	Syndrome	Associated Cancers								
		BR	OV	CO	EN	ME	PA	GA	PR	OC
BRCA1	Hereditary Breast and Ovarian Cancer Syndrome (HBOC)	●	●				○		○	
BRCA2		●	●			○	●		○	
MLH1	Lynch Syndrome / Hereditary Non-Polyposis Colorectal Cancer (HNPCC)		○	●	●		○	●		●
MSH2			○	●	●		○	●		●
MSH6			○	●	●		○	○		○
PMS2			○	●	●		○	○		○
EPCAM			○	●	●		○	●		●
APC	Familial Adenomatous Polyposis (FAP)/ Attenuated FAP (AFAP)			●			○	○		●
MUTYH	MUTYH-Associated Polyposis (MAP) Cancer Risk			●						●
CDKN2A (p16INK4A)	Melanoma-Pancreatic Cancer Syndrome (M-PCS)					●	●			
CDKN2A (p14ARF)	Melanoma Cancer Syndrome (MCS)					●	○			
CDK4						●	○			
TP53	Li-Fraumeni Syndrome (LFS)	●	○	○	○	○	○	○	○	●
PTEN	PTEN Hamartoma Tumor Syndrome (PHTS)	●		○	●					●
STK11	Peutz-Jeghers Syndrome (PJS)	●	●	●	●		●	●		●
CDH1	Hereditary Diffuse Gastric Cancer (HDGC)	●		○				●		
BMPR1A	Juvenile Polyposis Syndrome (JPS)			●			○	●		○
SMAD4	Juvenile Polyposis Syndrome (JPS) & Hereditary Hemorrhagic Telangiectasia (HHT)			●			○	●		●
PALB2	PALB2-Associated Cancer Risk	●					○			
CHEK2	CHEK2-Associated Cancer Risk	●		○					○	
ATM	ATM-Associated Cancer Risk	●					○			
NBN	NBN-Associated Cancer Risk	○							○	
BARD1	BARD1-Associated Cancer Risk	○								
BRIP1	BRIP1-Associated Cancer Risk	○	○							
RAD51C	RAD51C-Associated Cancer Risk	○	○							
RAD51D	RAD51D-Associated Cancer Risk		○							

● High Risk ○ Elevated Risk

Panel testing

Advantages

- Greater time and cost efficiency
- Greater sensitivity for cancer risk
- FH overlapping multiple syndromes
- Small families with limited information
- Assess risk in people not meeting criteria
- Examine moderate and high penetrance genes

Disadvantages

- Moderate risk genes-limited or nonexistent risk and management data
- Variants of uncertain significance (VUS)
- Mutations missed by NGS
- Complex pre-test counseling