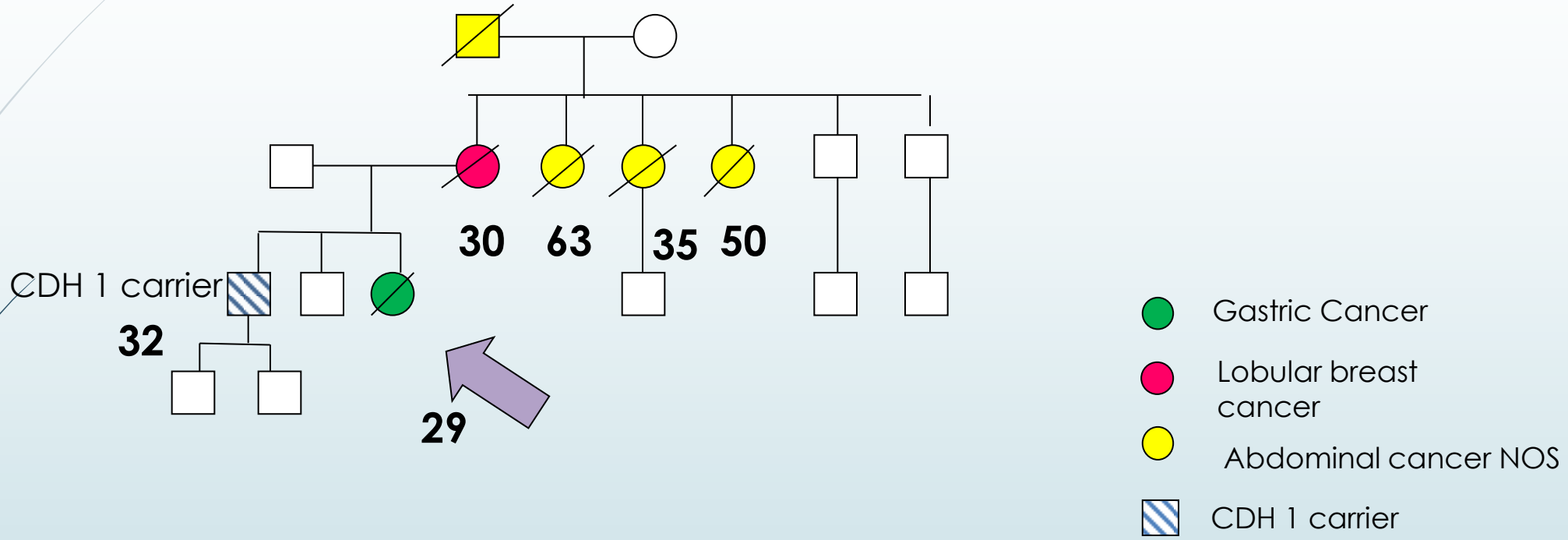
Germline testing for hereditary Cancer Syndromes Update 2015

Topics to be discussed

- Case Presentation
- Standard Indications for testing common syndromes
- Next Generation Sequencing and panel testing
- Unique Challenges to genetic testing
- Conclusions for clinical management today



Family with Gastric Cancer and CDH1 mutation

CDH positive

- Rare
- 80% risk of gastric cancer by age 80
- Gastric cancer is submucosal and not detected on endoscopy
- Young lobular breast cancer
- Autosomal dominant
- Now a common “incidental “ finding in genetic panel testing

Hereditary Susceptibility to Cancer

- Who to test
- What test to select, and when to update testing on previously tested families
- What are the cancer risks associated with the mutation and how accurate are the estimates
- What interventions are indicated
- Is there evidence supporting improved clinical outcomes

When Should Genetic Testing Be Considered?

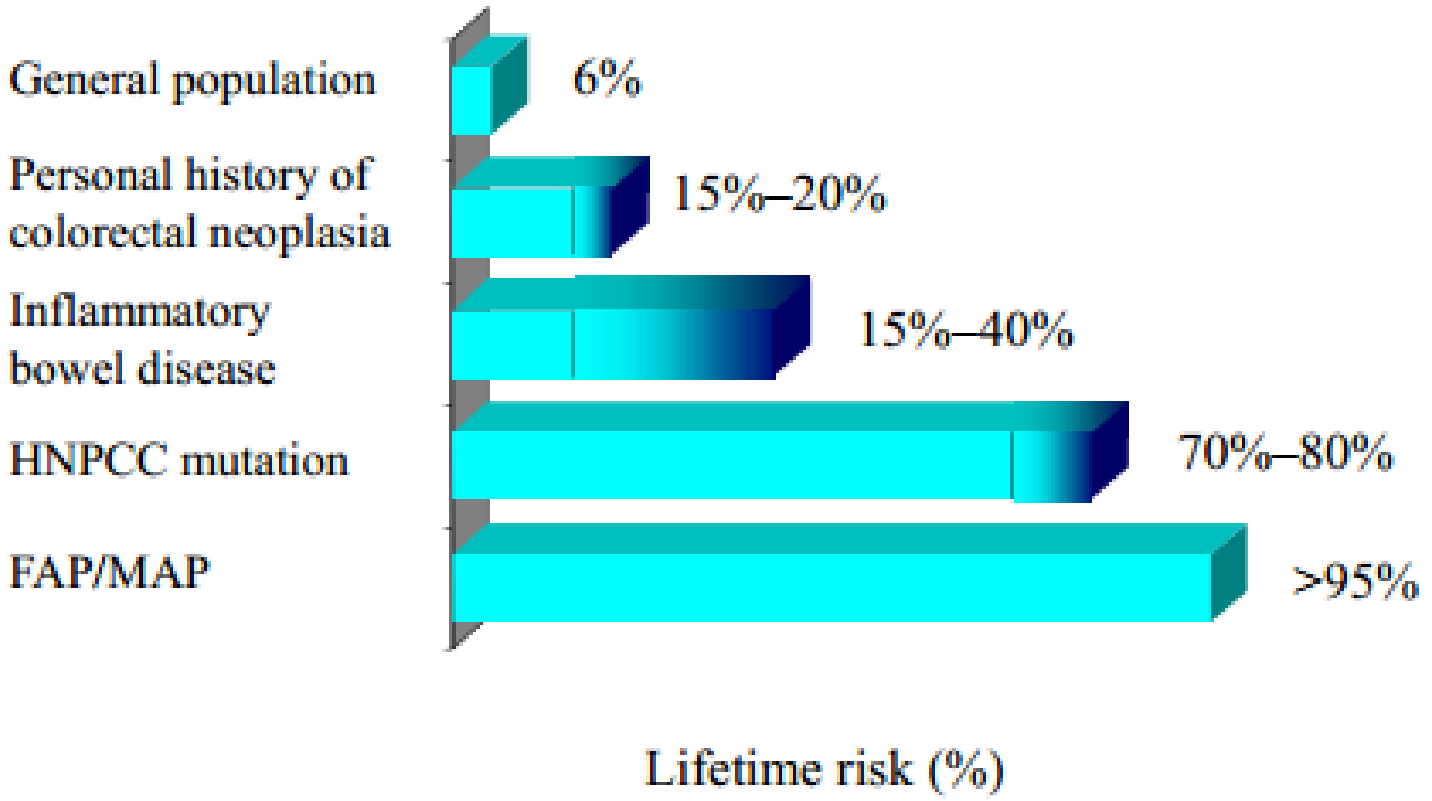
- Patient has a reasonable likelihood of carrying an altered cancer susceptibility gene
- Genetic test is available that can be adequately interpreted
- An affected individual is available for testing
- Results will influence medical management
- Patient wants information (empowerment)

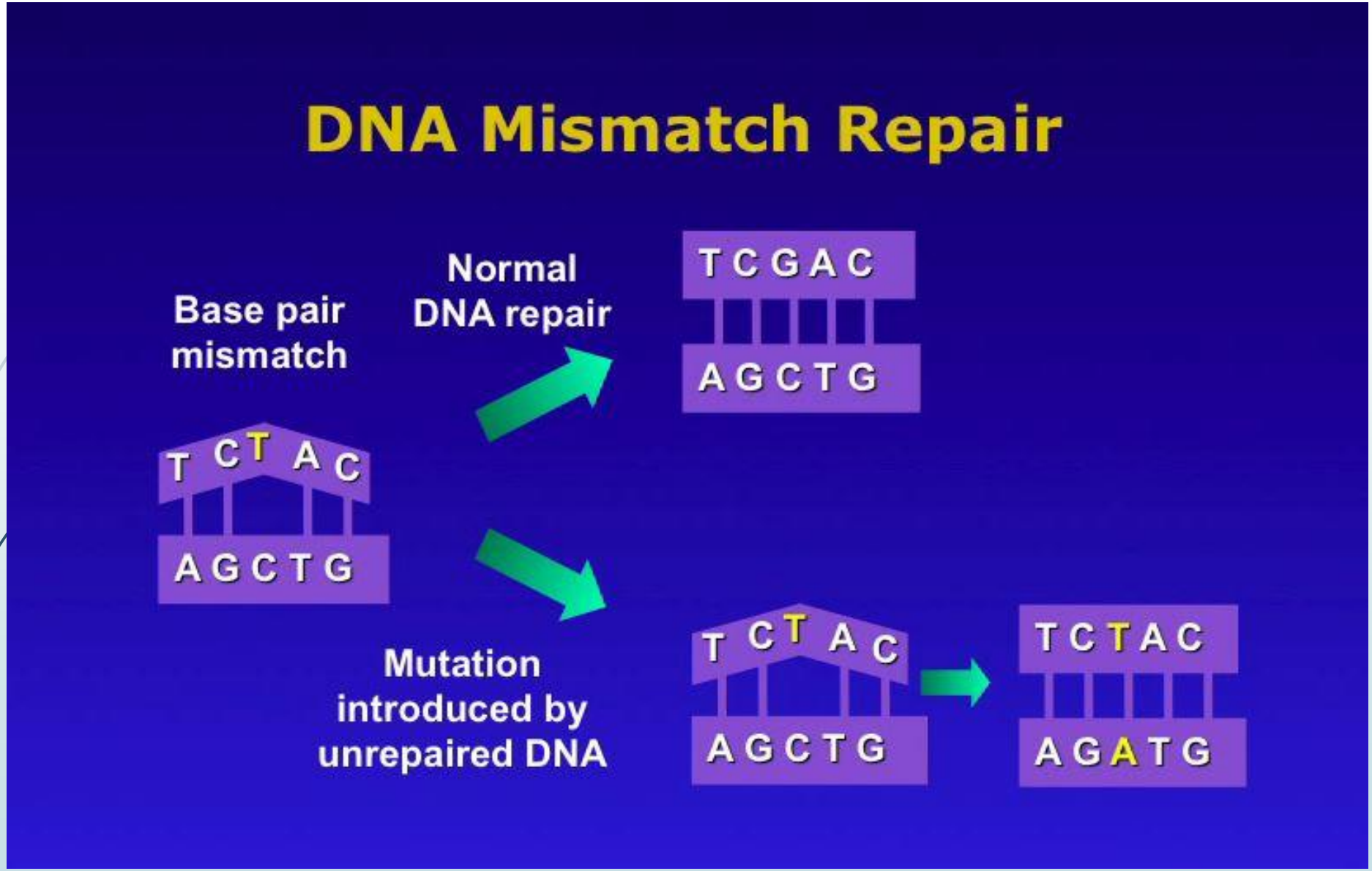
SYNDROME	Risk cancer	Recommendations
HBOC: **Hereditary Breast and Ovarian Cancer** BRCA 1 BRCA2	Breast 85% Ovary 40%	MRI and Mammogram age 25 Prophylactic oophorectomy
Hereditary Colon Uterine LYNCH	Colon 80% Uterine 40%	Colonoscopy yearly age 25 Gyn prophylactic surgery
Hereditary Pancreatic Cancer PALB2, BRCA2	60% Pancreas breast 40%	Research screening
Hereditary Gastric Cancer CDH	Gastric cancer 60-80%	Consider prophylactic surgery

Genetic testing in colorectal endometrial cancer families

- As common as BRCA
- Result can save lives with as much or more impact than BRCA
- Testing today in a fraction of the candidate patients

Risk of Colorectal Cancer

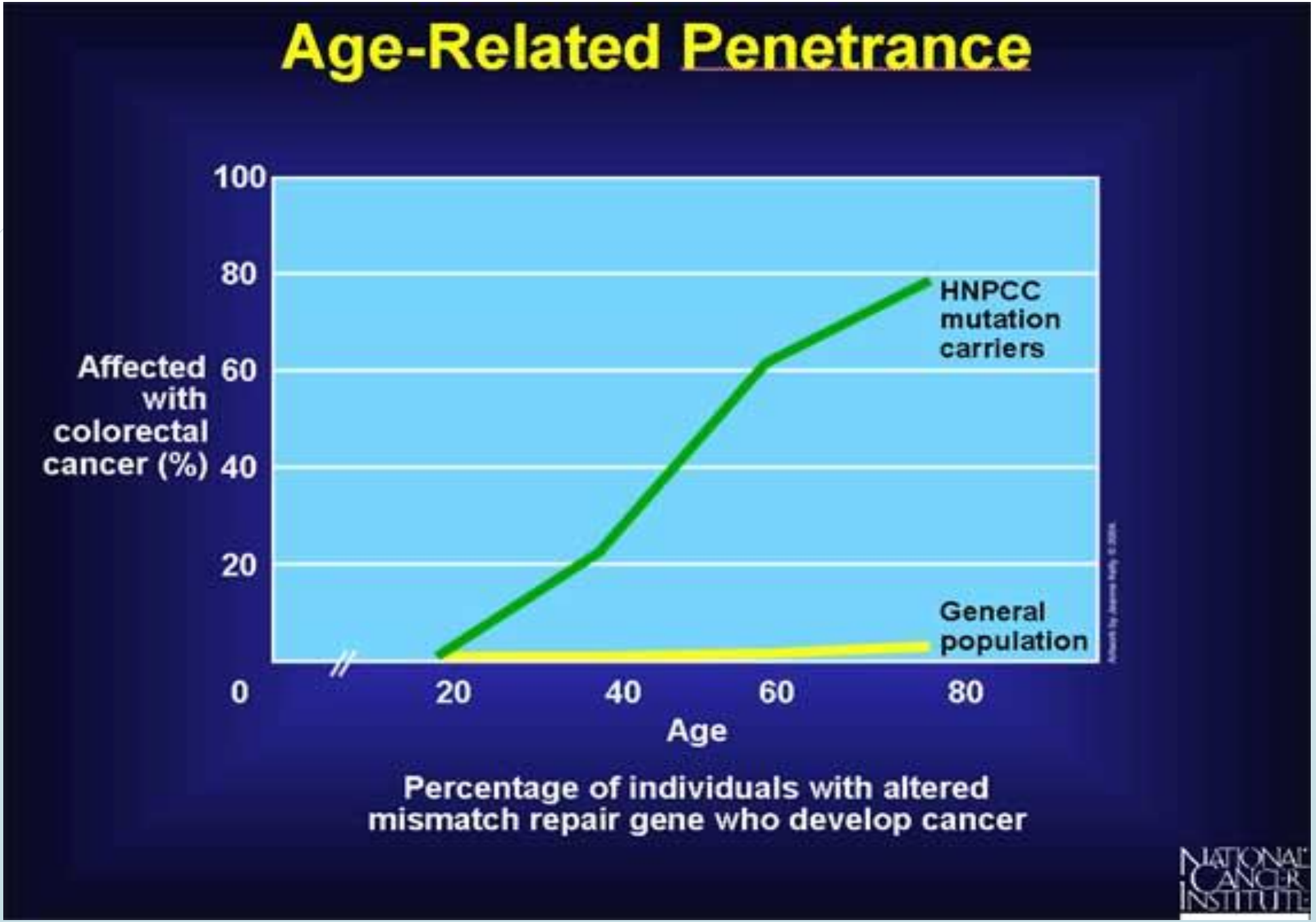



DNA Mismatch Repair
Base pair mismatch
T C T A C
A G C T G
Normal DNA repair
T C G A C
A G C T G
Mutation introduced by unrepaired DNA
T C T A C
A G C T G
T C T A C
A G A T G

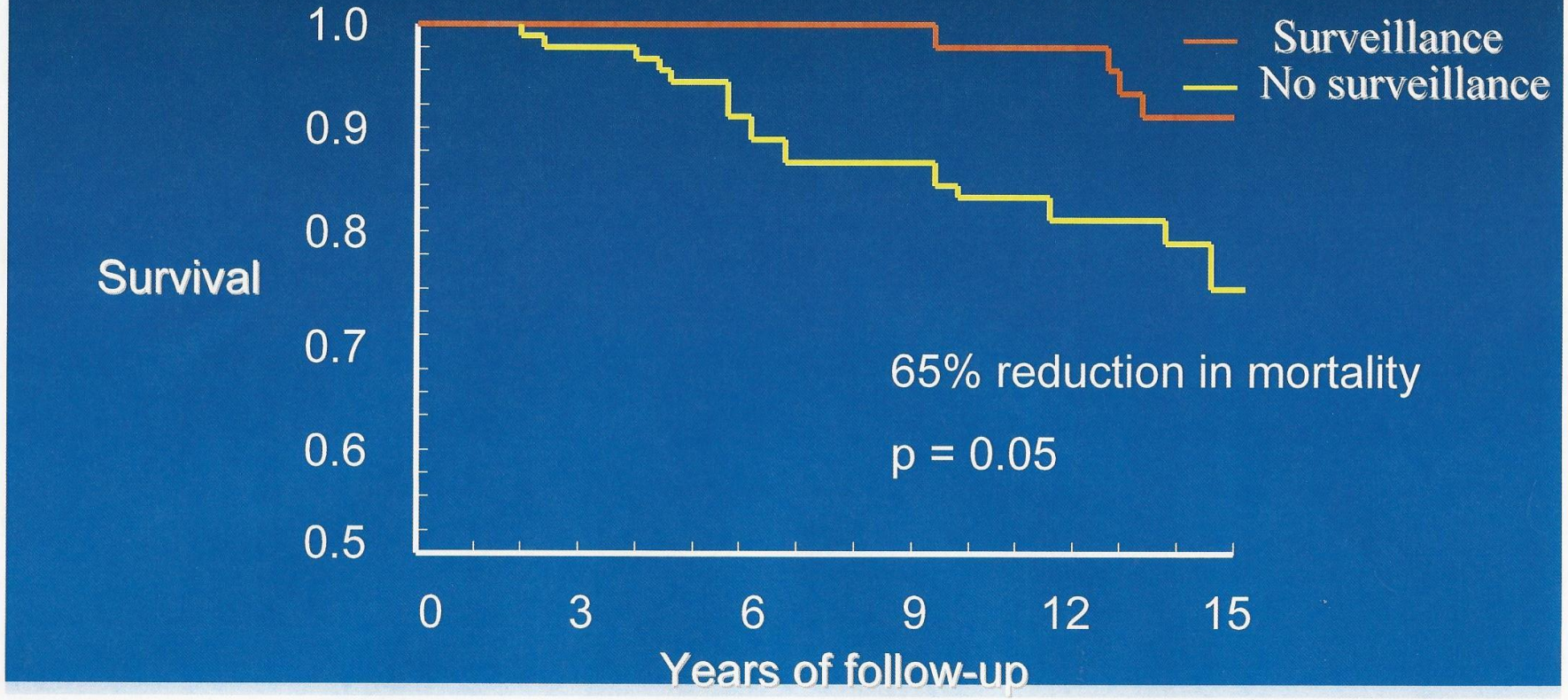
Testing Tumor for Lynch Syndrome

Tumor analysis-Screening for Lynch syndrome in the tumor (reliable in colon and uterine)
MSI-Microsatellite Instability
IHC-Immunohistochemistry

• Germline genetic testingDiagnostic test
MLH1, *MSH2*, *MSH6*, *PMS2*, *EPCAMM*

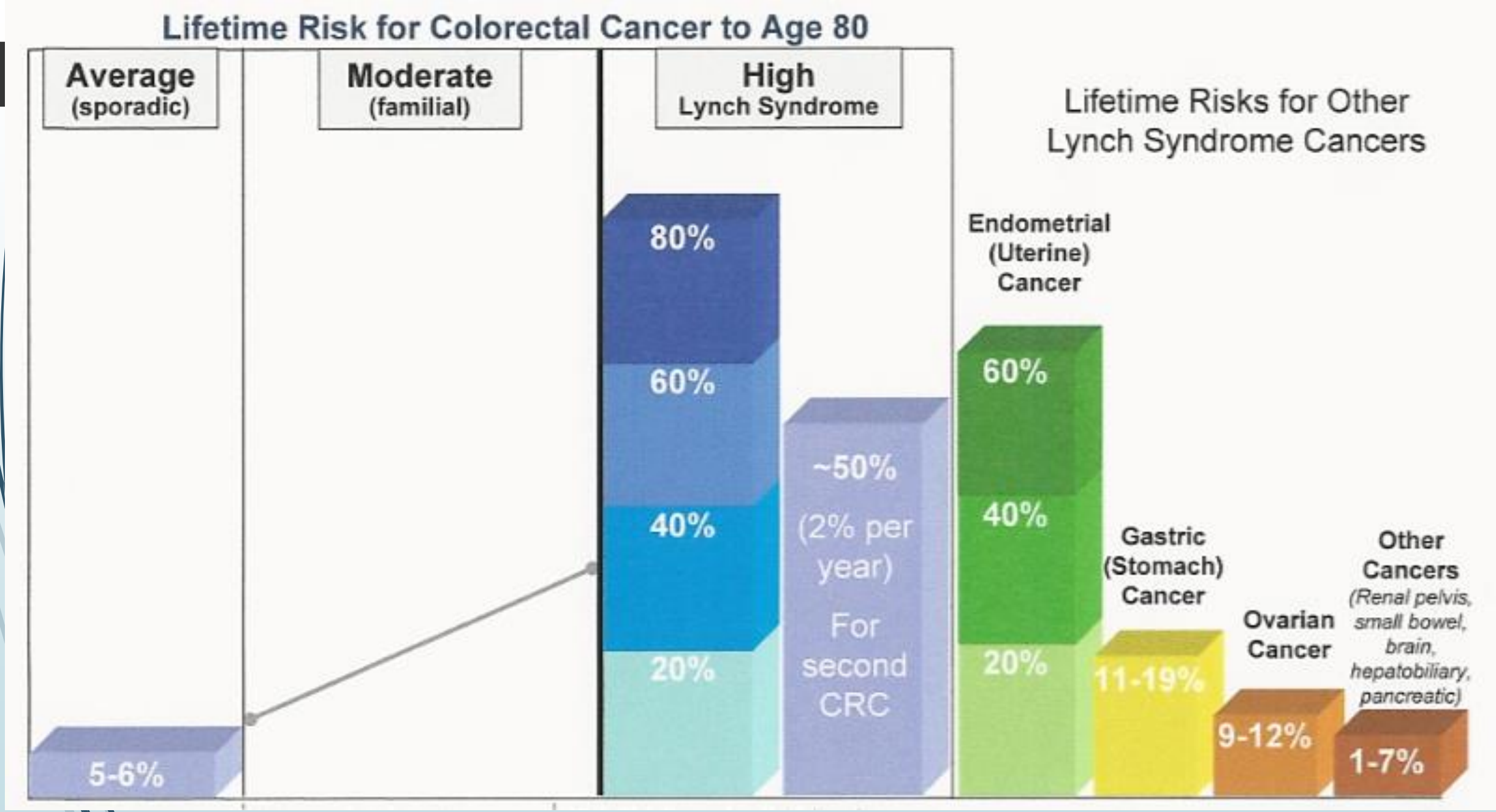

Age-Related Penetrance
Affected with colorectal cancer (%)
100
80
60
40
20
0
20
40
60
80
Age
HNPCC mutation carriers
General population
Percentage of individuals with altered mismatch repair gene who develop cancer
NATIONAL CANCER INSTITUTE

Surveillance Improves HNPCC Survival



Jarvinen HJ, et al. *Gastroenterology*. 2000;118:829-834.

City of Hope


Lifetime Risk for Colorectal Cancer to Age 80
Average
(sporadic)
Moderate
(familial)
High
Lynch Syndrome
5-6%
80%
60%
40%
20%
~50%
(2% per year)
For second CRC
Lifetime Risks for Other Lynch Syndrome Cancers
Endometrial (Uterine) Cancer
60%
40%
20%
Gastric (Stomach) Cancer
11-19%
Ovarian Cancer
9-12%
Other Cancers
(Renal pelvis, small bowel, brain, hepatobiliary, pancreatic)
1-7%

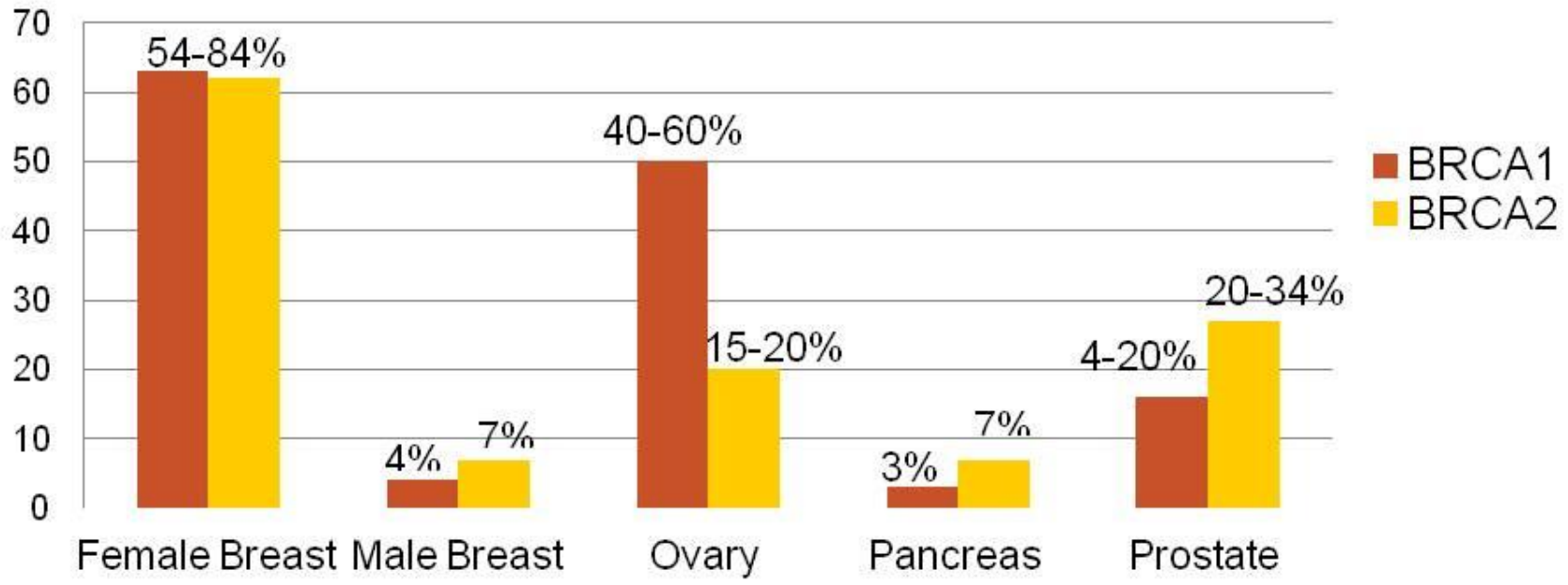
Hereditary Breast and Ovarian Cancer

- Breast cancer before age 50
- Ovarian cancer at any age
- Male breast cancer at any age
- Multiple primary cancers
- Ashkenazi Jewish ancestry
- Relatives of a *BRCA* mutation carrier
- Triple Negative under age 60

Science 2003;302: 643-6

www.nccn.org

Cancer Risks in Carriers of Gemline Mutations in *BRCA1* and *BRCA2*



PRESENTED AT: ASCO Annual '15 Meeting

Management Guidelines *BRCA1/2* Carriers

Management Option	Screening Interval/Comments
SCREENING	
• Clinical Breast Exam • Breast MRI • Mammogram	• Q6-12 mos beginning age 25 • Yearly age 25-75 (then individualize) • Yearly age 30-75 (then individualize)
• Transvaginal ultrasound* • CA-125*	• Q6 mos beginning age 30 • Q6 mos beginning age 30
PREVENTION	
• Bilateral mastectomy • Bilateral salpingo-oophorectomy	• Discuss option with patient • Recommend by age 35-40 and when childbearing complete
• Consider oral contraceptive • Consider tamoxifen	

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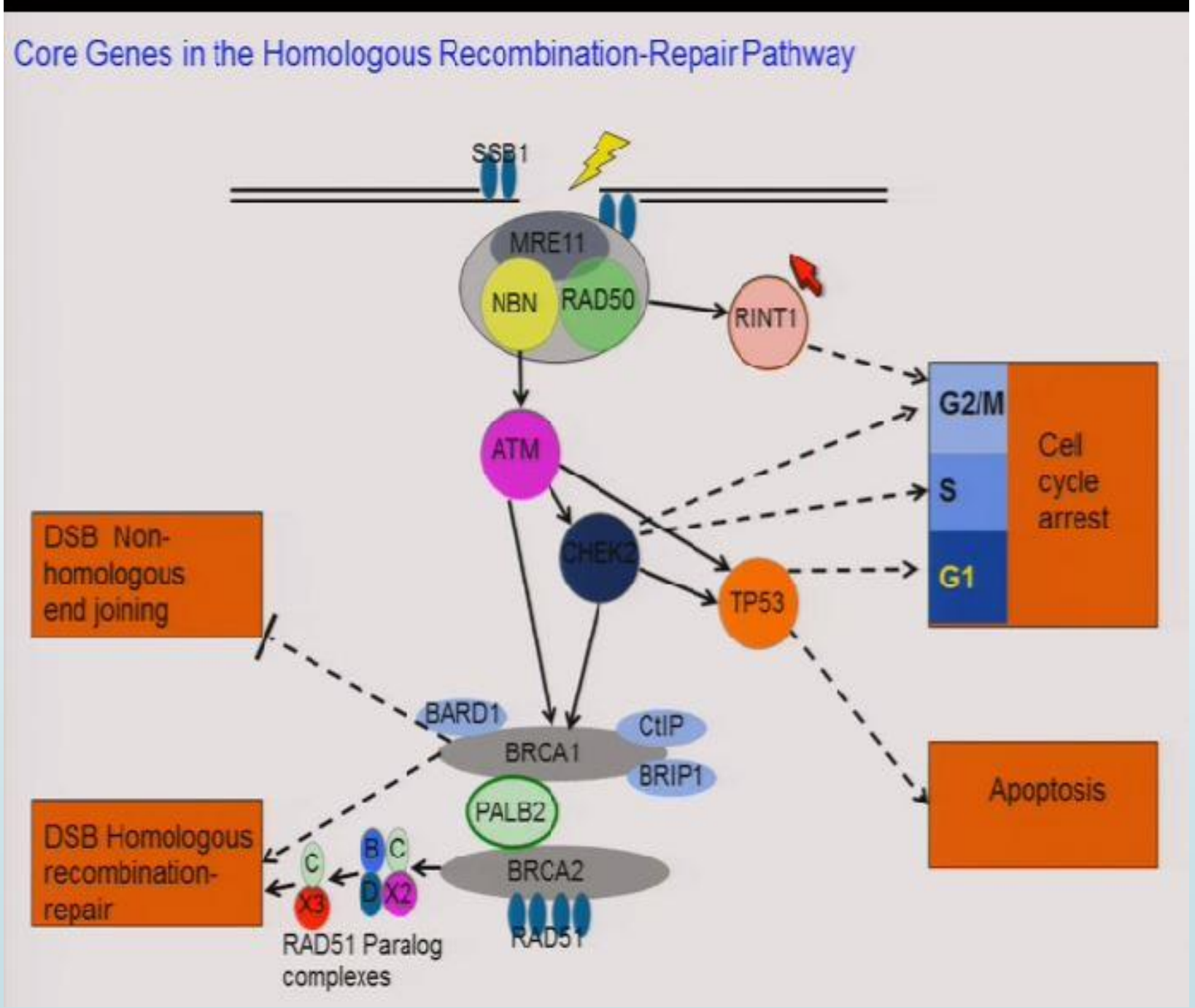
Updates in germline testing for breast and ovarian cancer families

- Large deletion and duplication testing
- Palb2
- Risk modifiers in Gene carriers
- Panel testing*

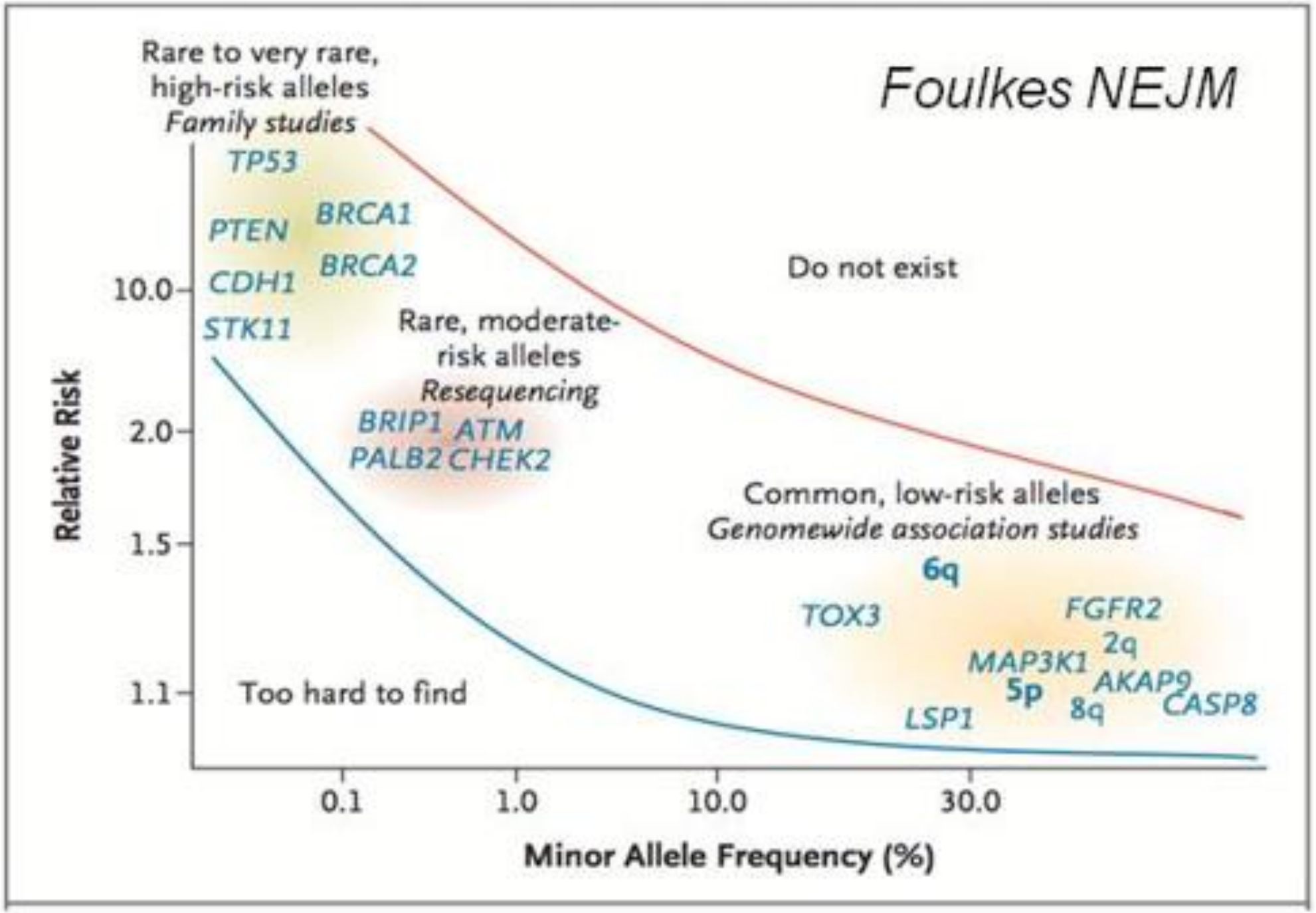
Large Duplication and Deletion analysis

- BART
- Not done on most patient prior to 2012:Routine sequencing: detecting single base changes, by Sanger sequencing
- rearrangement test: detects all large deletions and duplications of BRCA 1 and BRCA 2 coding: available since 2006, not routing
- LRP: 5-site large rearrangement panel in BRCA 1 available since 2002, routinely included
- Identifies additional 2.3% overall, accounts for upto 9% of all mutations in high risk families


Core Genes in the Homologous Recombination-Repair Pathway
SSB1
MRE11
NBN
RAD50
RINT1
ATM
CHEK2
TP53
G2/M
S
G1
Cel cycle arrest
DSB Non-homologous end joining
BARD1
CtIP
BRCA1
BRIP1
PALB2
BRCA2
RAD51
Apoptosis
DSB Homologous recombination-repair
C
X3
B
C
D
X2
RAD51 Paralog complexes

PALB-2

- Partner and Localizer of BRCA 2
- Homologous DNA repair
- Risk of Breast Cancer overall is 33% by age 70
- Risk is increased to 58% if family history greater than two first degree relatives
- accounts for 2.4% of familial aggregates of breast cancer
- Associated with increased risk of pancreatic cancer


Foulkes NEJM
Rare to very rare, high-risk alleles
Family studies
TP53
PTEN
BRCA1
BRCA2
CDH1
STK11
Do not exist
Rare, moderate-risk alleles
Resequencing
BRIP1
ATM
PALB2
CHEK2
Common, low-risk alleles
Genomewide association studies
6q
TOX3
FGFR2
2q
MAP3K1
5p
AKAP9
8q
CASP8
LSP1
Too hard to find
Relative Risk
10.0
2.0
1.5
1.1
0.1
1.0
10.0
30.0
Minor Allele Frequency (%)

Breast Cancer Risk Factors

Nulliparity/Late Parity	RR 1.3
Early Menarch/ Late Menopause	RR 1.3
Alcohol Abuse	RR 1.3
Hormone Replacemant Therapy	RR1.4
Obesity	RR 1.5
Female Age 65	RR1.7
Family History	RR 1.7
Dense Breast Tissue	OR 4.3
Biopsy with Atypia or LCIS	RR 5.0

Breast Cancer and Ovarian Cancer and Panel Testing

- Testing has become clinically available on a widespread basis for only about 1-2 years
- Most labs offer wide variety of panels
- First data are recently published on results of panel testing
- Issues of VUS and estimates of cancer and incidental findings are common to all cancers
- Which panels, and what are the main issues
- What are the results in Breast, ovarian cancer in early use of testing

Gene V Panel V Exome v Genome

	Single Gene	Gene Panel	Exome	Genome
Target Size	5000	500,000	50,000,000	3,000,000,000
Method	Sanger sequence	Next Gen Seq	Next Gen Seq	Next Gen Seq
Gene Targets	Selected	Selected	No selection bias	No Selection Bias
Incidental Findings	No	No	yes	Yes
Estimated Variants	0-3	50	100,000	38,000,000

NEJM 2015 Panel sequencing Breast Cancer Risk

Gene	RR	Absolute risk	Breast and other	comments
BRCA1	11.4	75%	Breasts ovary	BOADICA model
BRCA2	11	76%	Breast ovary prostate pancreas	
TP53	105	Est 40%	Multiple cancers: sarcoma, adrenal brain	Li Fraumeni
PTEN		unknown	Thyroid, uterine, clinical phenotype	Cowden's syndrome very rare
CDH1	6.6	53%	Gastric lobular breast	Diffuse gastric syndrome
STK11			Stromal tumors multiple cancers	Peutz Jegher Very very rare

NEJM 2015 Panel sequencing Breast Cancer Risk

Gene	RR	Absolute Risk	Breast and other cancer	comments
PALB2	5.3	45%	Ovary unclear, pancreas	
ATM	2.8	27%	Pancreas modest	
CHEK2	2.7	29%	Possible	100Del c RR is only 1.3
NBN	2.7	23%	Biallelic associated leukemia	c.657del5 Slavic founder is data
MRE, RAD51	2.5	>20%		

Growing List of labs offering panel testing in genetics

- Ambry
- ApolloGen
- ARUP
- GeneDX
- Invitae
- ColorMe
- Myriad
- Pathway Genomics
- Prevention Genetics
- Quest
- University of Washington
- Fulgent Diagnostics

Is there concordance between labs?

- Validations not published by all labs
- Validation requirements not standardized
- Extent of analysis may differ (e.g. intronic depth)
- Argument in favor of increased regulatory oversight

Mutations

- Silent
- Missense: change in amino acid
- Truncating:
- Deletions, insertions = frameshift
- Splice site mutations alter donor or acceptor splice site causes incorrect splicing of exons
- Non sense: stop codon TAG, TAA TGA

Types of Mutations

Normal Message:

THEBIGREDDOGRANOUT
THE BIG RED DOG RAN OUT

Deletions:

THE BIR EDD OGR ANO UT

Insertions:

THE BIG REB DOB GRA NOU TS

Variants of Unknown Significance

- THE BIG RED DOG RAN OUT

- THE BIG NED DOG RAN OUT

Making Sense of a VUS

1. Amino acid conservation (charge, polarity, volume, hydrophobicity, etc.) and Grantham Matrix Score (GMS)
2. Prevalence in a control population (SNP?)
3. Co-segregation with disease in affected families
4. Location within gene and protein functionality
5. In silico analysis (Align-GVGD, PolyPhen, SIFT, NNsplice)
6. Evolutionary conservation
7. Concurrent with known deleterious mutations
8. LOH of wild type allele in tumors
9. Locus-specific databases (BIC, LOVD, InSiGHT, MMR Genes Variant Database, IARC TP53 Database)

When should we test for a panel of cancer genes?

- This is a question that is not yet answered.
- When the results are actionable
- When the results can help families understand
- Mostly it is for future generations
- Only real standard test is in targeted gene
- Can be more cost effective if multiple genes are possible
- Consent for this information is complex

Panel Testing Results for Familial Breast Cancer

Genes	N = # Patients with deleterious mutation (%)	95% Confidence interval
Any deleterious mutation	11 (10.4)	5.30, 17.81
BRCA1 or *BRCA2*	7 (6.6)	2.70, 13.13
BRCA1	4 (3.8)	1.04, 9.38
*BRCA2**	3 (2.8)	0.59, 8.05
Other genes related to breast cancer	5 (4.7)	1.55, 10.67
*ATM**	2 (1.9)	0.23, 6.65
CHEK2	1 (0.9)	0.02, 5.14
PALB2	2 (1.9)	0.23, 6.65

* One patient had a deleterious mutation in both *BRCA2* and *ATM*

Conclusions on testing in breast cancer

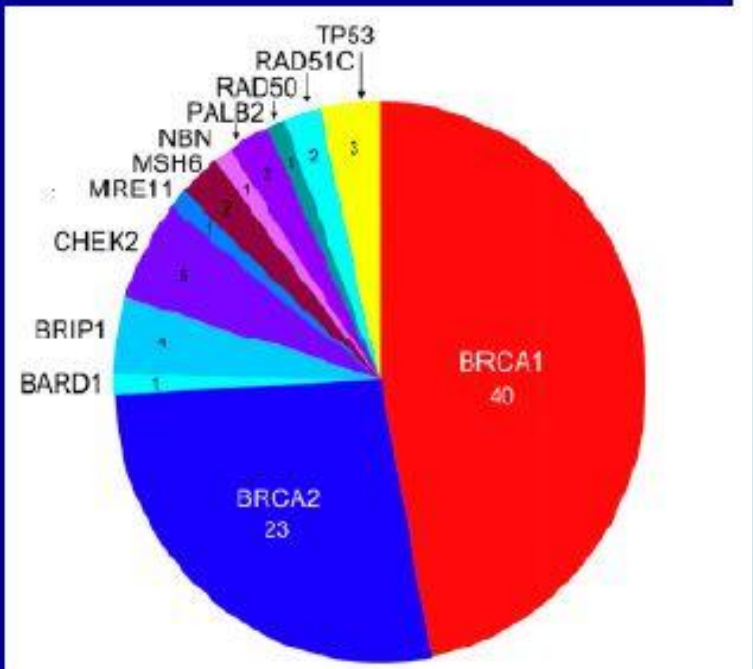
BRCA testing including deletions, Palb2 standard proven utility

Panel testing might identify a mutation in 10% of strong family history with modest unknown value

Most patients will have some unknown variant at this time

Germline Analysis of 12 DNA Repair Genes in Women with Ovarian Cancer

- 360 women unselected for age and family history
  - 273 ovarian, 48 peritoneal, 31 FT, 8 synchronous endometrial & ovarian
- **24%** germline mutation
  - Loss of function
  - >2/3 in BRCA1 or BRCA2
  - 12 genes represented
- Of women with mutation:
  - 30% had no family history
  - 37% ≥ 60 years old at diagnosis



Walsh T et al. , PNAS, November 2011:108;10832-18037

Genetic testing in Ovarian Cancer

- When testing for family history that includes ovarian cancer, panel testing may be indicated
- In part due to poor screening option for early detection
- And Effectiveness of prophylactic salpingo oophorectomy

Resources

- NCCN Guidelines
- ACMG American College of Medical Genetics
- GeneReviews list specific mutation
- http://www.ncbi.nlm.nih.gov/

National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2015
Genetic/Familial High-Risk Assessment: Breast and Ovarian

BREAST AND OVARIAN MANAGEMENT BASED ON GENETIC TEST RESULTS[a]

	Recommend MRI[c] (>20% risk of breast cancer[d])	Recommend RRSO	Discuss Option of RRM
Intervention Warranted based on gene and/or risk level	*ATM* *BRCA1* *BRCA2* *CDH1* *CHEK2* *PALB2* *PTEN* *STK11* *TP53*	*BRCA1* *BRCA2* Lynch syndrome[e]	*BRCA1* *BRCA2* *CDH1* *PTEN* *TP53*
Insufficient evidence for intervention[b]	*BARD1* *BRIP1*	*BARD1* *BRIP1* *PALB2* *RAD51C* *RAD51D*	*ATM* *BARD1* *CHEK2* *PALB2* *STK11*

When to consider panel testing

- Consider recommending panel testing: Suspect multiple syndromes, ovarian cancer
- Palb 2 should be included in BRCA testing
- For triple positive;P53, strong breast cancer CHEK 2, ATM so consider panel if including these
- Costs may drive testing , consumer drivers such as Color me
- Patient counselling is complex
- Be prepared for VUS, average 1-2 per test
- Germline testing for targeted therapies
- Somatic testing may also drive germline discovery

Conclusion

- Germline genetic testing is standard of care for high risk individuals when test results will change patient management
- Best established in breast, ovarian cancer, and lynch syndrome
- Panel testing is readily available
- Panel testing results in identification of additional gene testing when patients undergoing testing are BRCA negative about 10% of the time.
- Risk estimates are likely to be variable based on the clinical context
- The availability of genetic testing data is being made available of the interpretation data and precise estimates are necessary to identify true clinical value
- Gene testing of tumors and panel testing also mean an large portion of the population will encounter results they had not expected without counseling. Education and research are key.

BOCA RATON
REGIONAL HOSPITAL

EUGENE M. & CHRISTINE E.
LYNN CANCER INSTITUTE

MORGAN PRESSEL CENTER FOR CANCER GENETICS

561-955-GENE

561-955-4363