

Genetics of cancer in EGAT study



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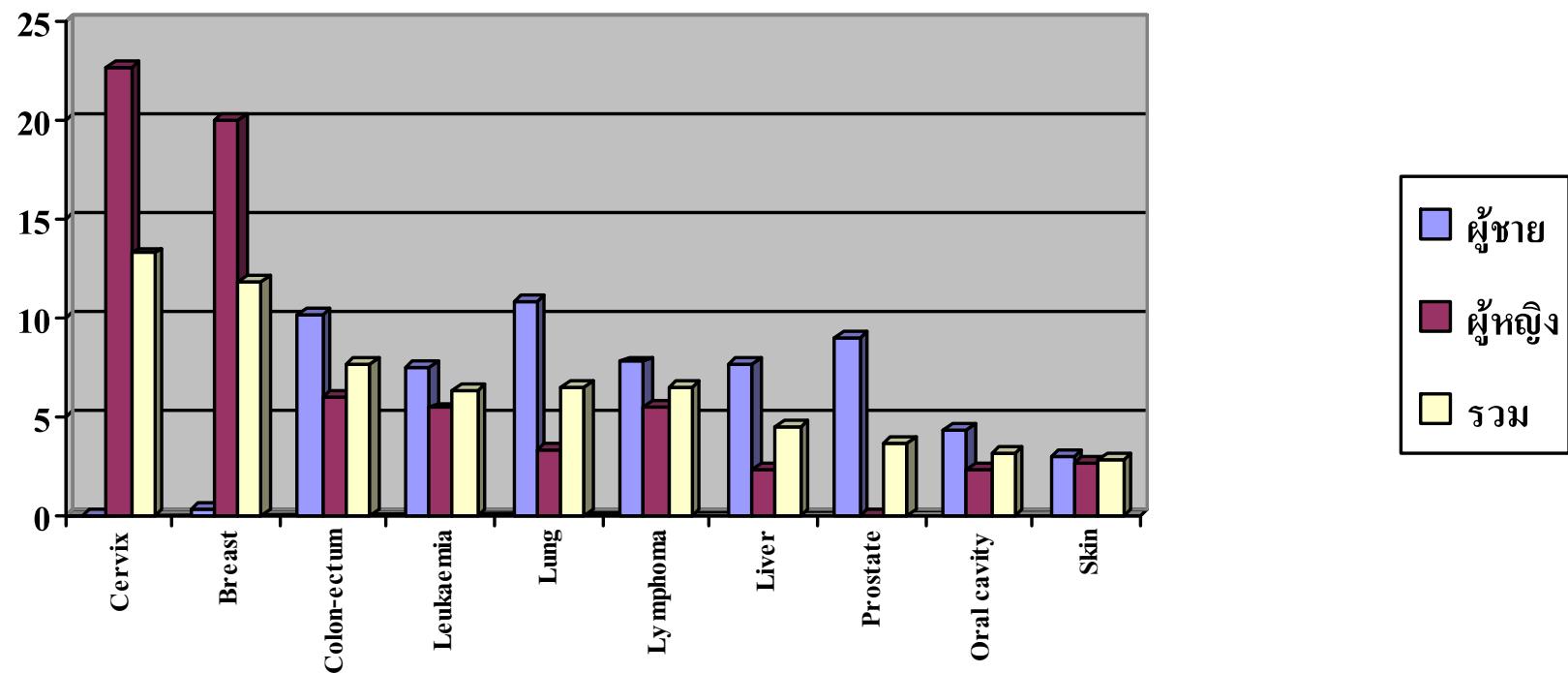
*Ramathibodi Hospital, Mahidol University
Bangkok, Thailand*

- **Cancer develops because of a complex mix of environmental and genetic factors.**
- **For some:-**
 - Environmental factors pose the greatest risk
- **For others:-**
 - It is inherited susceptibility
- **For most:-**
 - Combination of all the above.

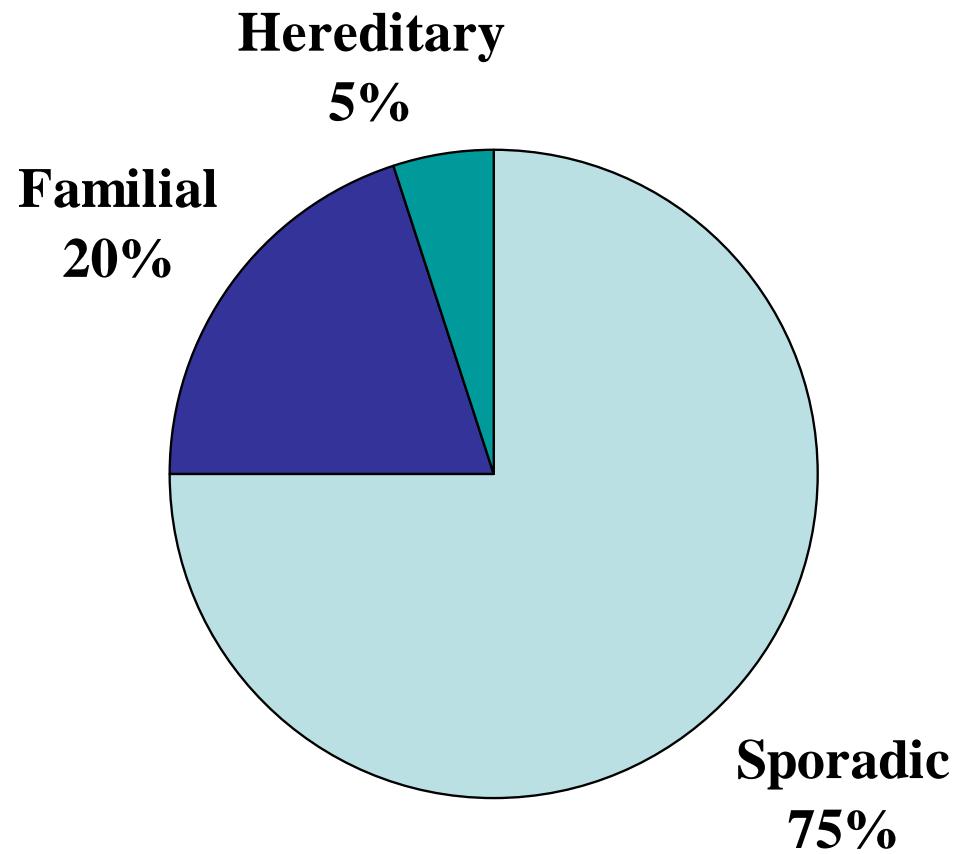


The leading sites of cancer in Thailand

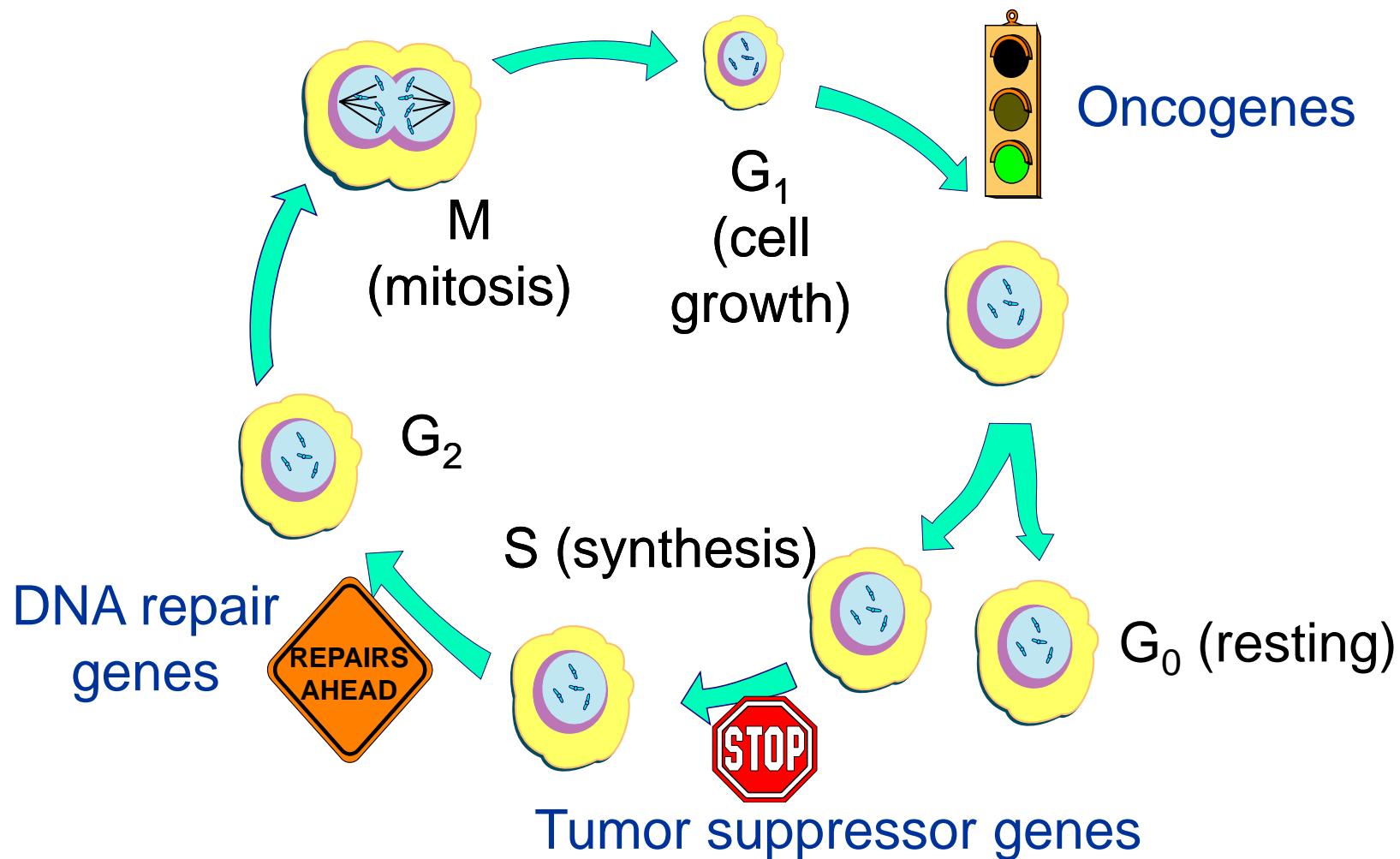
Ministry of Health 2005



Etiology of Cancers

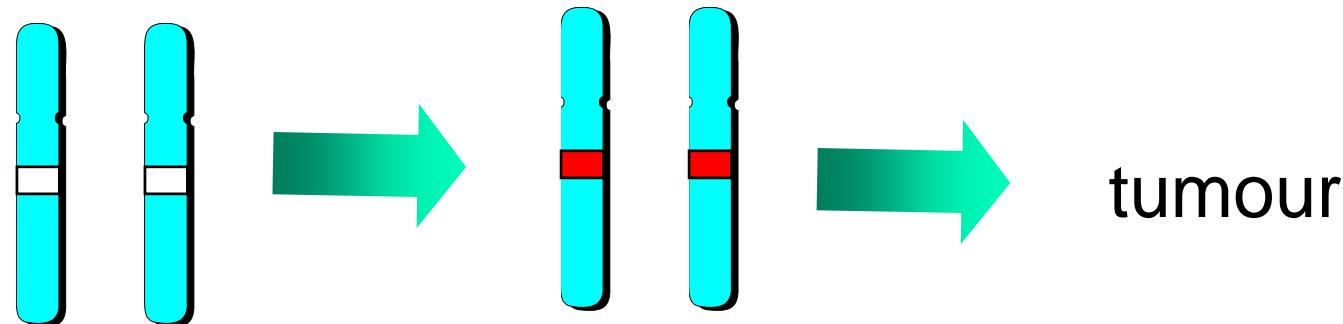


The Cell Cycle



Cancers Arise From Gene Mutations

in genes protecting **against** cancer

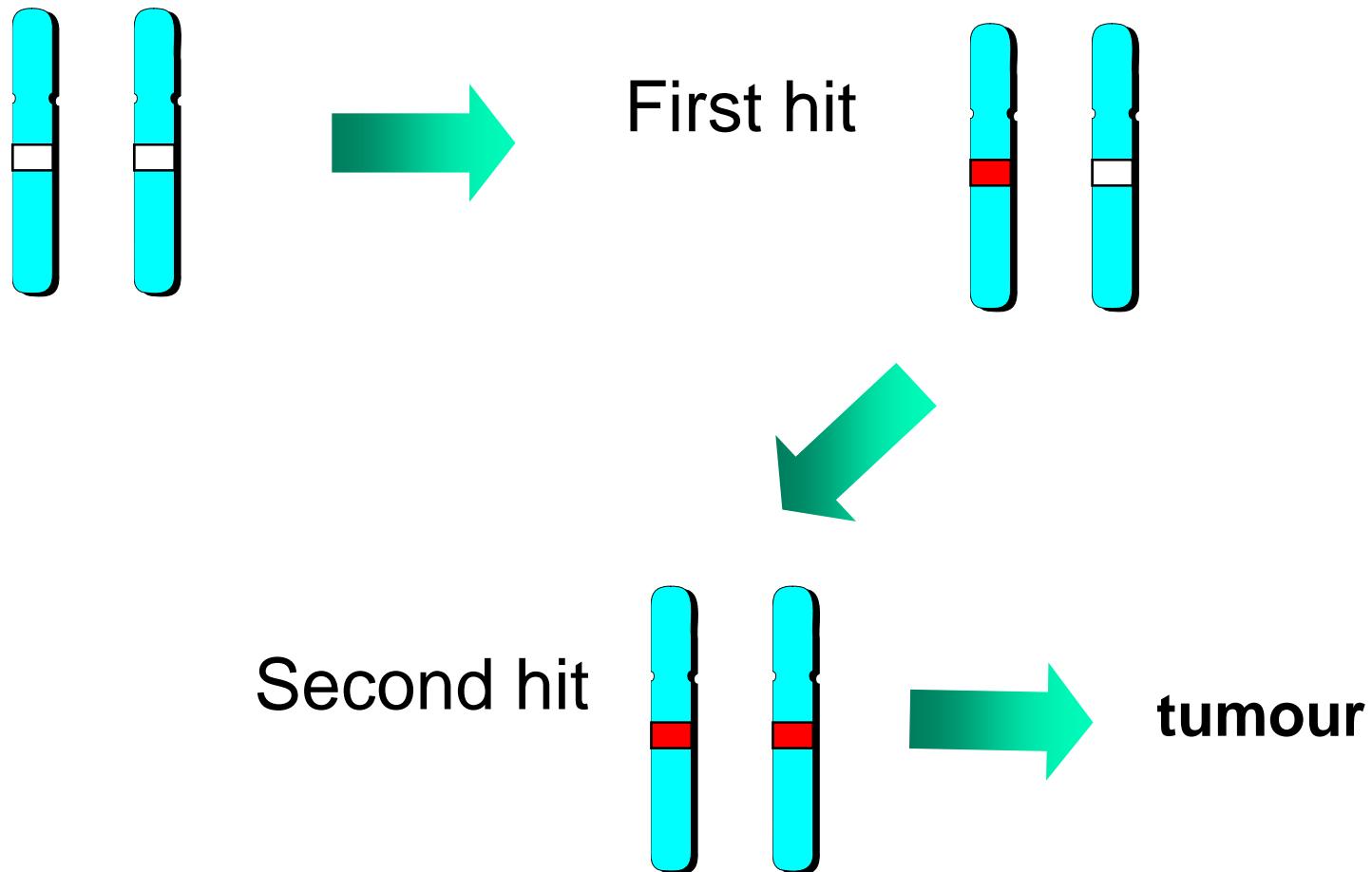


All cancer is genetic

BUT

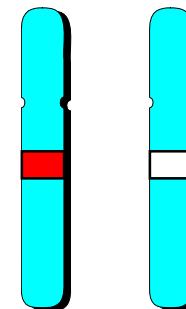
not all cancer is inherited!

Knudson's 'Two-Hit Hypothesis' (Somatic Mutation)

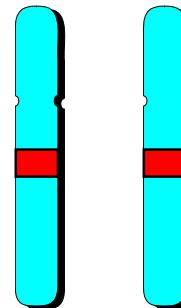


Knudson's 'Two-Hit Hypothesis' (Germline Mutation)

First hit is in
germline

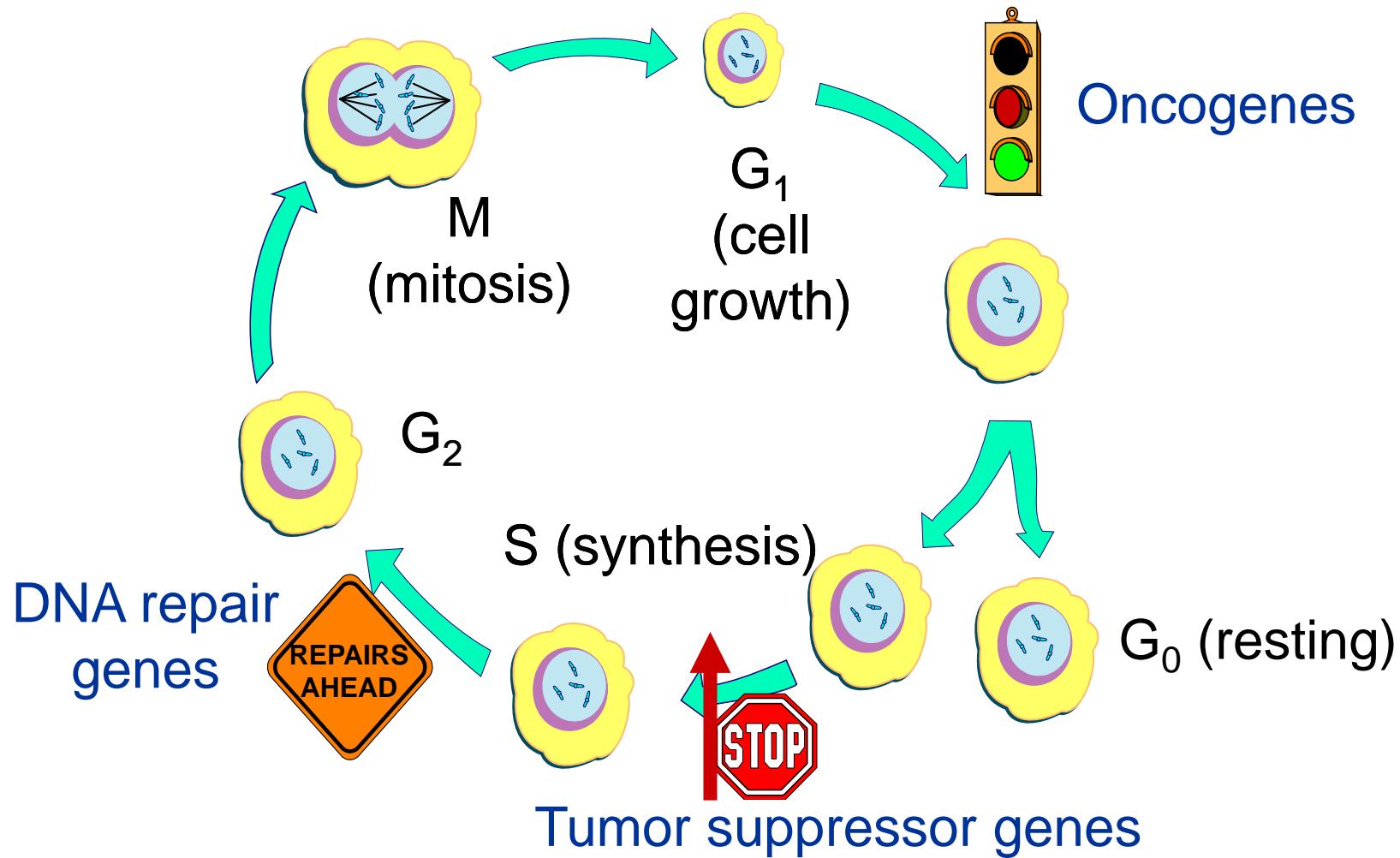


Second
hit is
somatic

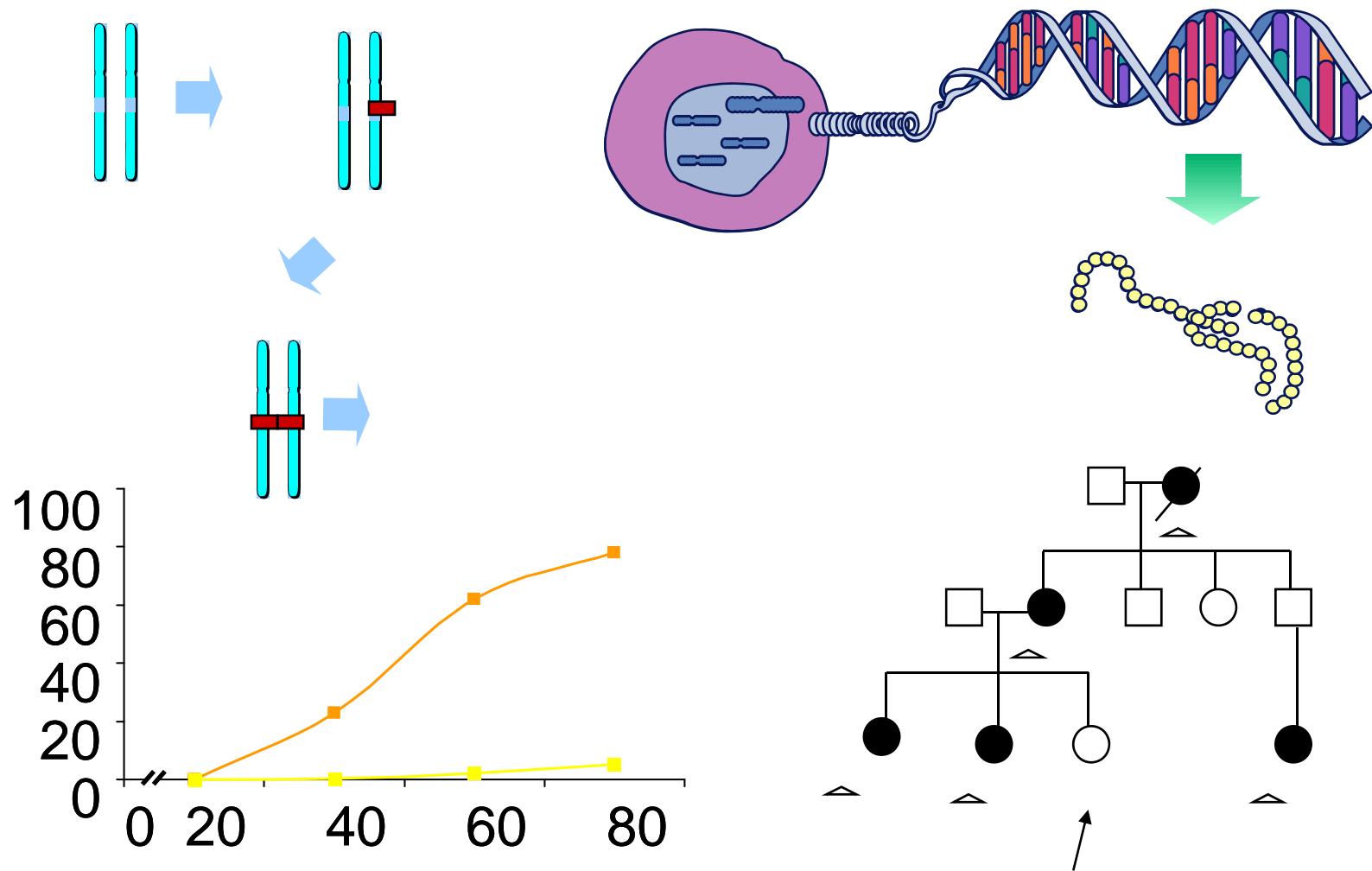


tumour

The Cell Cycle



In summary...



Pattern of cancer

	Skin	GI tract	Male genital tract	Female genital tract	Endocrine	Nervous system	Musculoskeletal	Urinary tract	Haematological
BRCA1	•			•					
BRCA2	•		•	•					
HNPCC	•	•		•		•		•	•
FAP (APC)	•	•			•	•	•		
PJS		•	•	•	•			•	
Hereditary gastric (ECAD)		•							
LFS	•	•			•	•	•	•	
VHL					•	•		•	
Cowden	•	•		•	•	•	•	•	
Gorlin	•					•			
RB	•					•	•		
MEN1		•			•	•			
MEN2					•				
NF2	•					•			
Fumarase carrier				•					



Population of EGAT study

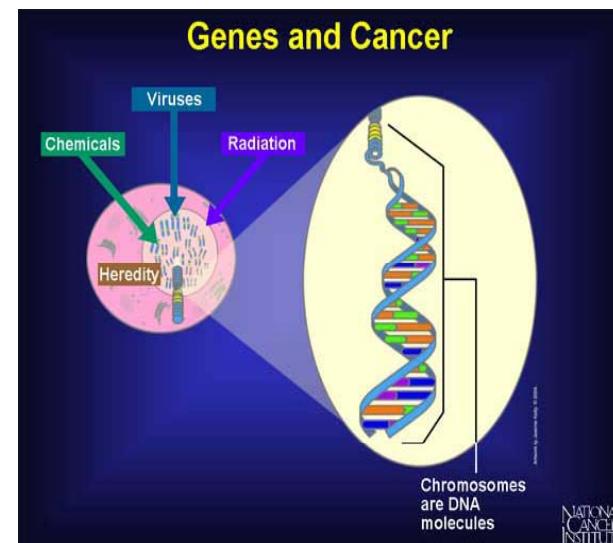
- EGAT 1 ~ 2,800
- EGAT 2 ~ 2,200
- EGAT 3 ~ 2,000



พลิตไไฟฟ้าเพื่อความสุขของคนไทย

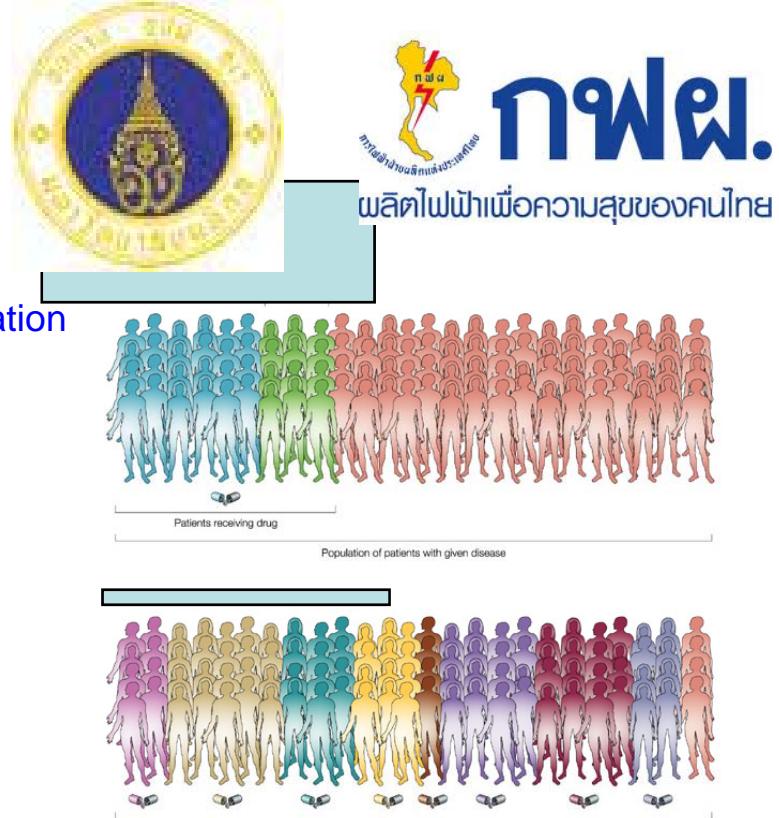
Cancers ~ 680

Cancers ~ 572
Breast, Colon, others



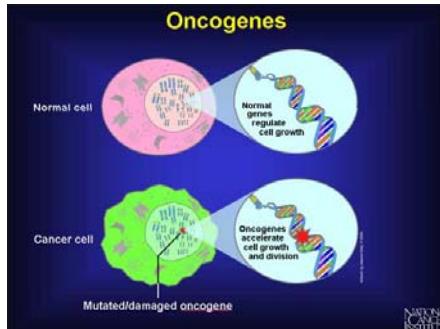
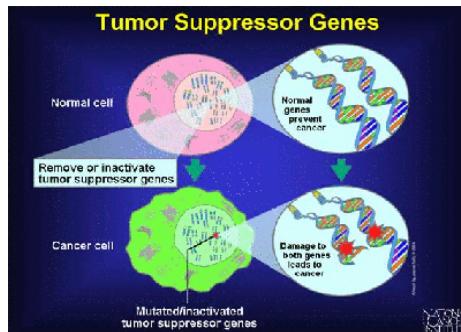
Outcome Prediction

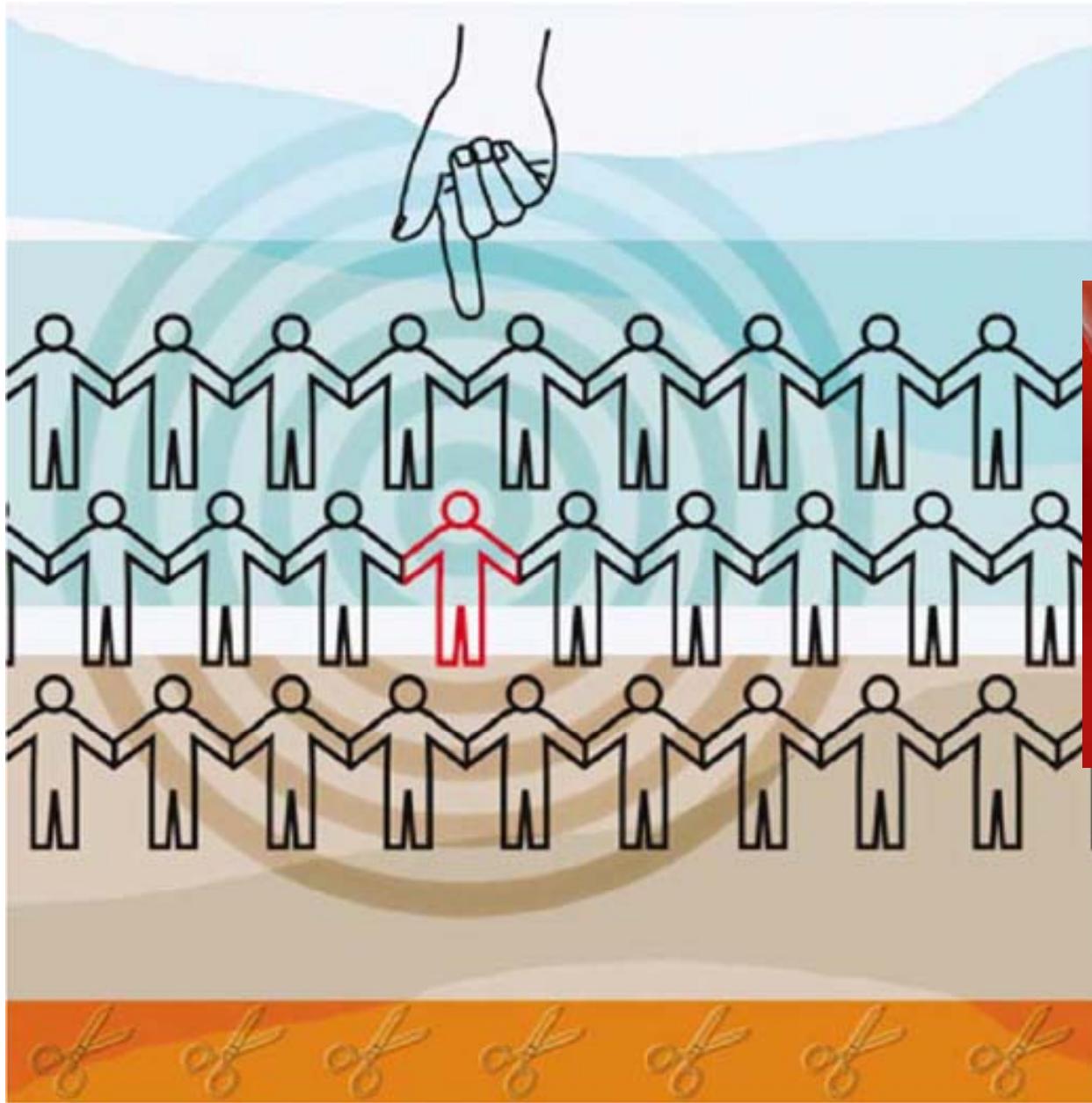
1. Identified group of cancer genes in EGAT population
2. Identified individual 's risk for cancer prevention
3. Apply these genetic markers to Thai population
4. Appropriate surveillance screening
5. Compromise health economics



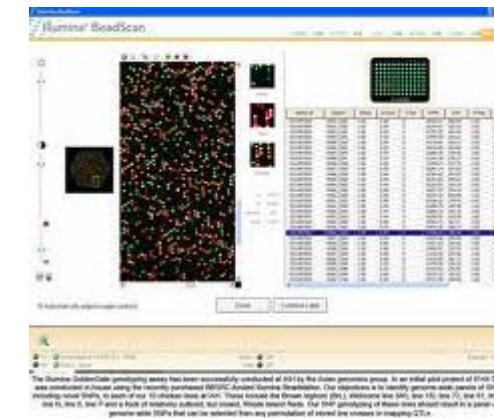
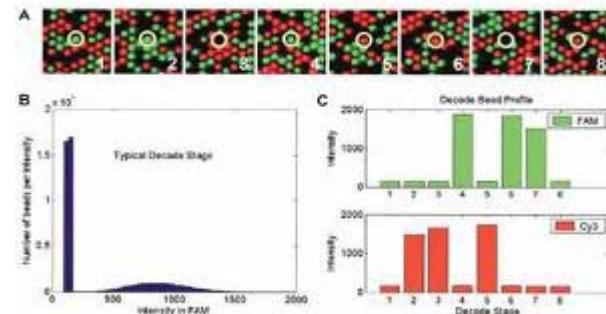
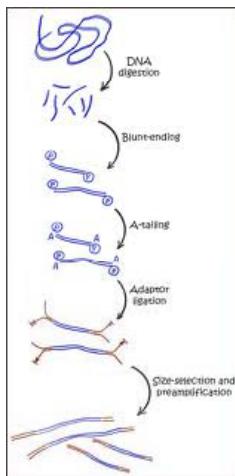
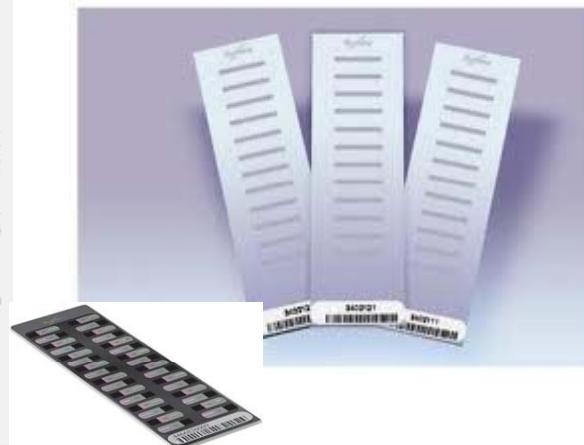
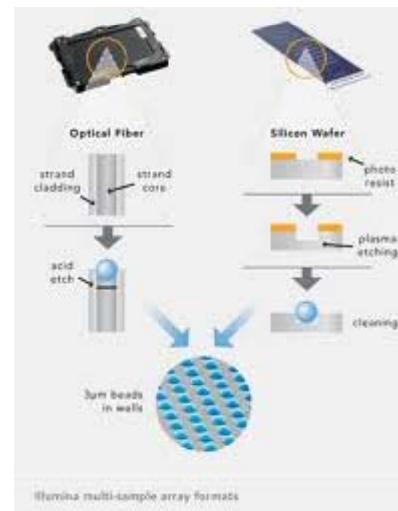
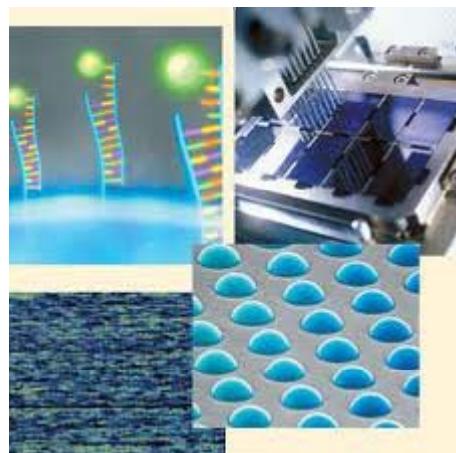
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CCCAGGCAGTCATGATTCAAGGCTCACGAGTCACATGACTGCCGTATTTGTCTCTGTGCTGTCACCA
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GAGAACATAAGAGAGGCAAAACTATCATTCTCATCTGAAAGCCAGTACTCACCAGCAAATTAGGCAC
ATCATAGGCTTAGAACCAAGAAAATCTGAGTTAACTAGTGATAAAATGGATAGTAAATTCCGAATG
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AGCCTGTGGATGAAGTAACTAGATCTCAAGCAGTCATGAGATGTGGAAAGACAGCCAAGCCTCCCACCT
ATAAGTCATAGAAAACATTCTACATGGATTGAGATTGAGATTATGCATTACACATTCAACAAAAAT
TAAGTTAGTGCCTACCACATGTTGAGCATT **TTCTCGGCACTGATATGGACC** TGTGACCAAAACAGGCCT
AATCCCTATATATGGTCTATGAAGAGATCAATAAAGCAAGCAAATAAGAAATAAATAAAAAGAGA
ATTGTGAAAATTAGTATCAACAGGACACTGTGATGAAAAAACACAGAACCCCTACTTTAGATAACTTTAT
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GCTGTTATTAA **TTCTCGGCACTGATATGGACC** TGTGACCAAAACAGGCCT
CGGCCACATTCTATTAAAAAATTGC **TTCTCGGCACTGATATGGACC** TGTGACCAAAACAGGCCT
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TTGAGGTTGAGCTGAGGTTGAGCTTAAAGCCCTCAGTGAACAGATA **TTCTCGGCACTGATATGGACC** TGTGACCAAAACAGGCCT
TCCAGGTAAGGCTGAGGTTGAGCTTAAAGCCCTCAGTGAACAGATA **TTCTCGGCACTGATATGGACC** TGTGACCAAAACAGGCCT
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CACTGGGAATCACAGGAATTGATGAGGTTGAGCTTAAAGCCCTCAGTGAACAGATA **TTCTCGGCACTGATATGGACC** TGTGACCAAAACAGGCCT
AAACAACCCATAAGTGCTCTGCTGAAACAAACAAACAAACCCCCCTCAAGTTATCAGTGTAAAAT
TTGGCTTACATTCTGGACAATGGTGGAGTTACCCATCTGCTACTTAGAATGTTACAGAAAT

- cIllumina's Cancer SNP Panel candidate gene-based genotyping platform
- GoldenGate genotyping assay
- Illumina SNPs 1,421 ตัวแหน่งที่อยู่ใน SNP500 Cancer database [2] ซึ่งเป็นส่วนหนึ่งของ
- Cancer Genome Anatomy Project
- National Cancer Institute
- case-control association study
(proto-oncogenes) และยีนที่เพิ่มความเสี่ยงต่อโรคมะเร็ง
- (tumour-suppressor genes หรือ cancer-susceptibility genes อื่นๆ)
- [2] ข้อดีของ SNPs ในฐานข้อมูลนี้คือเป็น SNP ที่พยาบาลเลือกมาโดยให้อยู่ภายในยีนหรือถ้าไม่ชั้นนั้นก็อยู่ใกล้กับยีนมาก
- และโดยเฉพาะอย่างยิ่งพยาบาลเลือก SNPs ในยีนที่มีลักษณะ non-synonymous
- ซึ่งน่าจะส่งผลดีต่อการคิดโรคโดยตรงมากกว่าจะเป็นเพียง linked polymorphic markers ท่านั้น





Illumina technique

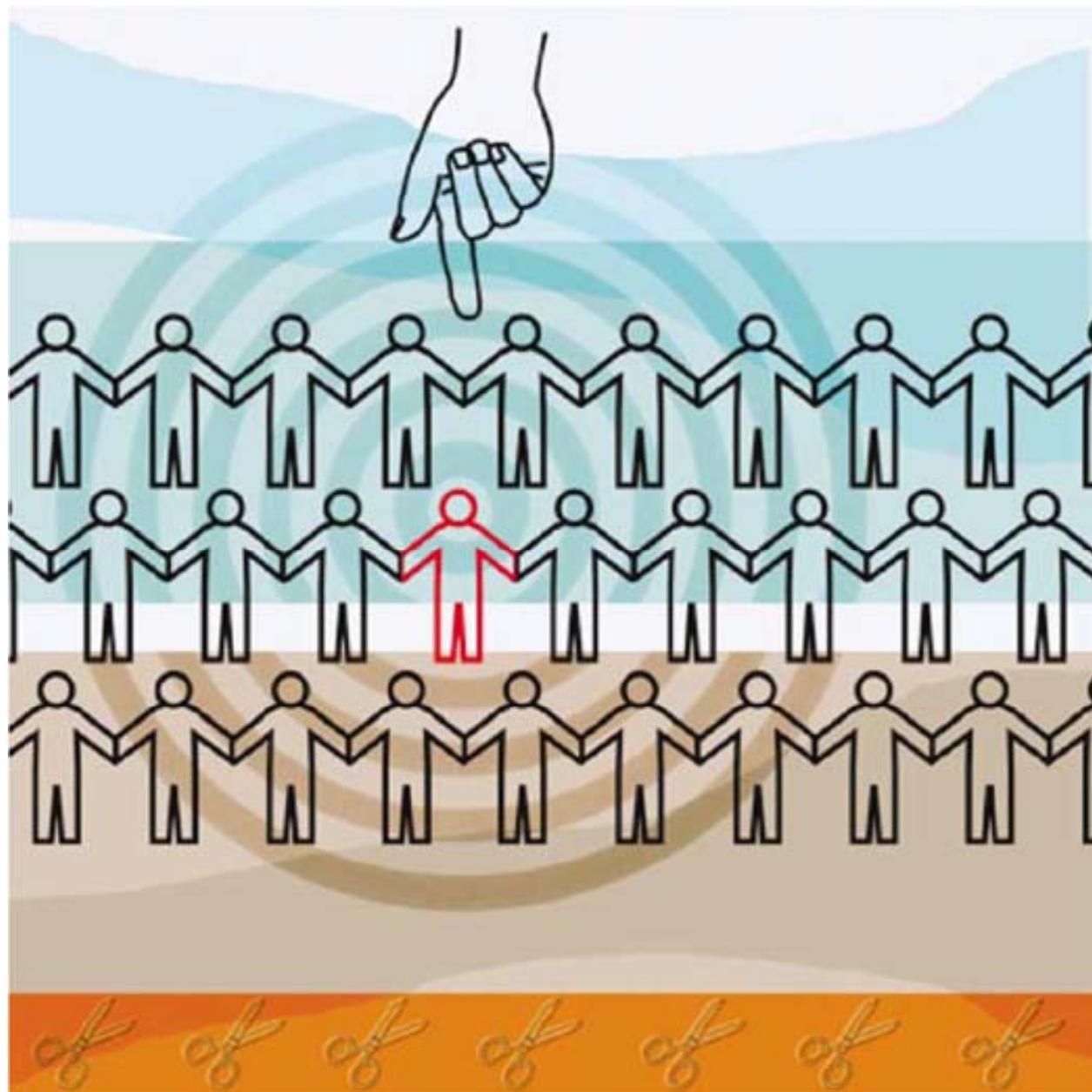




Colon cancer

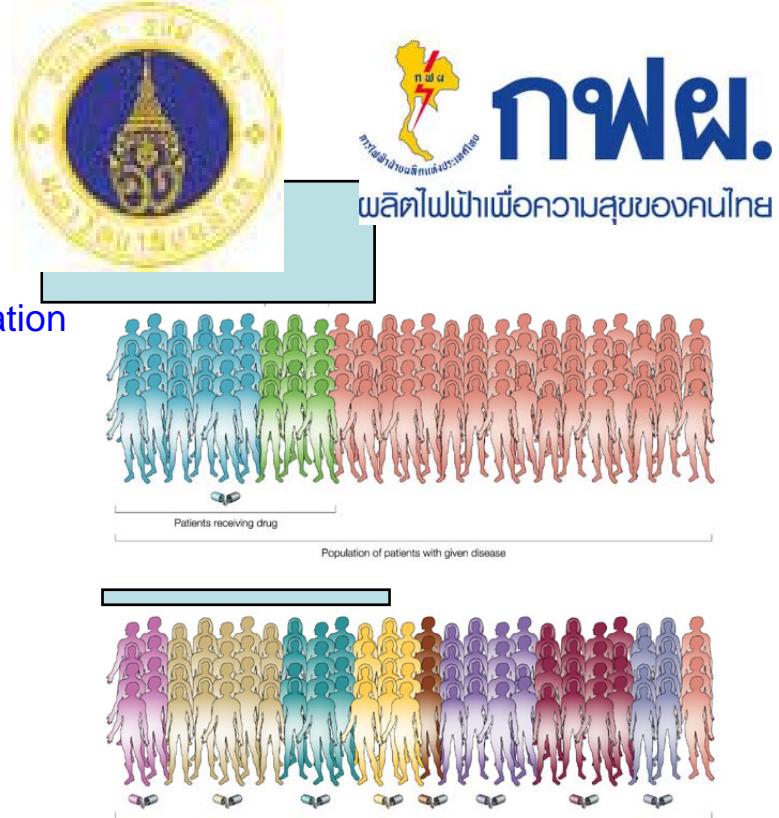
<i>Informative SNPs</i>	= 178
<i>1st correction</i>	= 93
<i>2nd rearrangement</i>	= 23
<i>3rd validation</i>	= 19





Outcome Prediction

1. Identified group of cancer genes in EGAT population
2. Identified individual 's risk for cancer prevention
3. Apply these genetic markers to Thai population
4. Appropriate surveillance screening
5. Compromise health economics

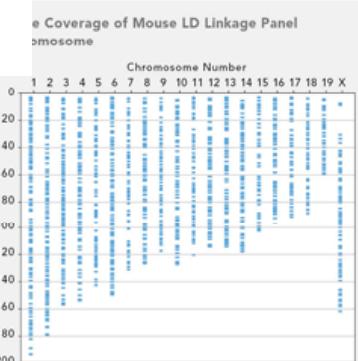
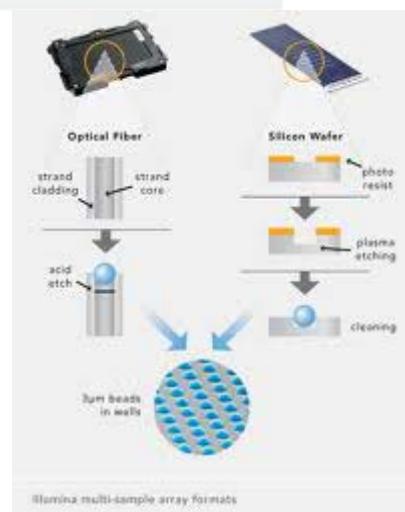
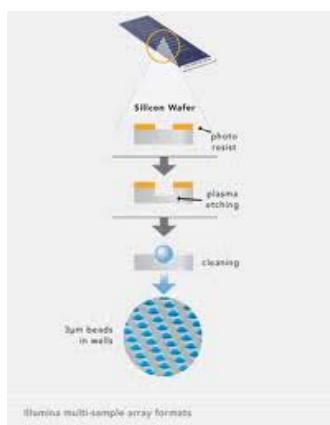
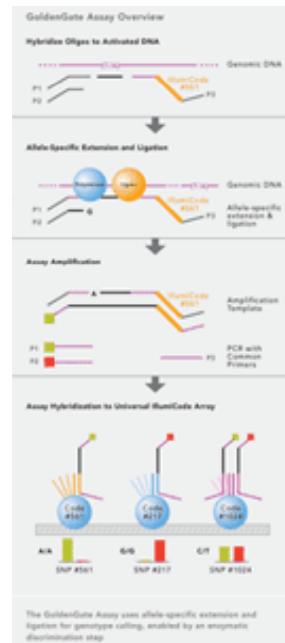
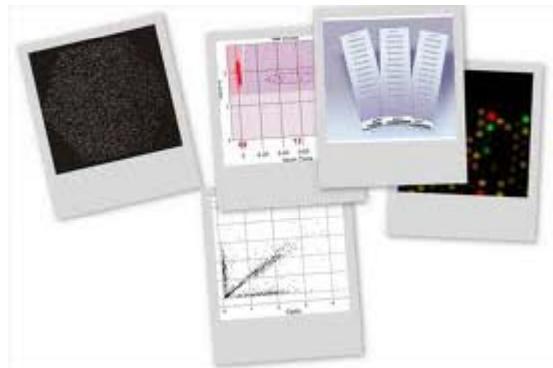


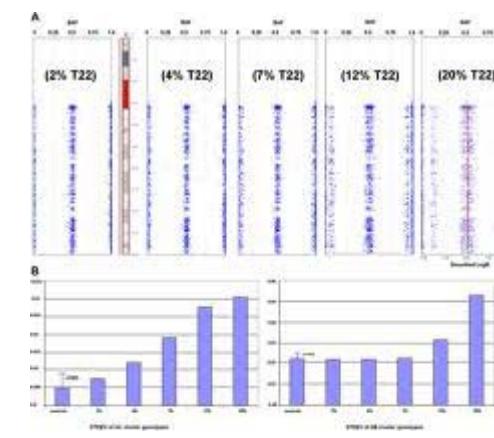
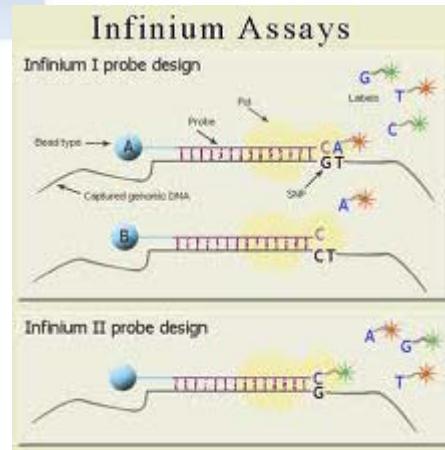
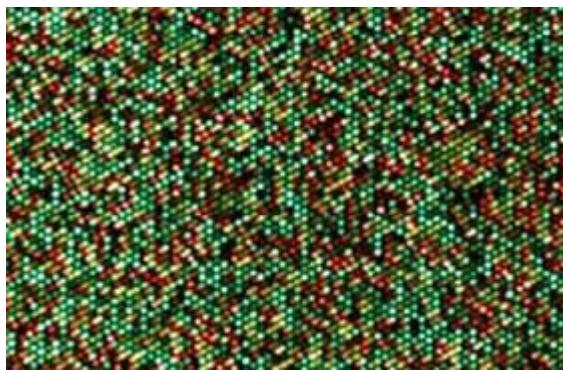


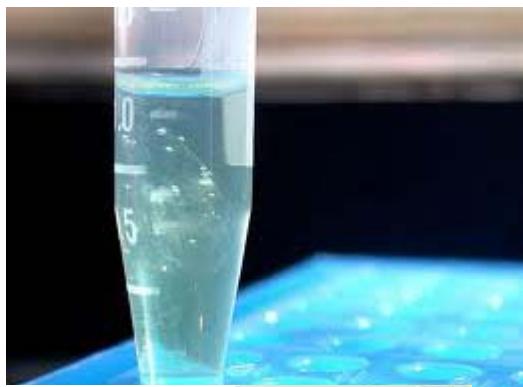
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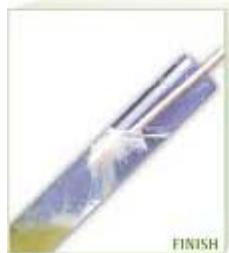






STEP 4

What is that Stringy Stuff?



FINISH

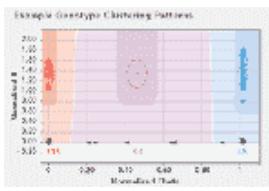


www.shutterstock.com - 35020663

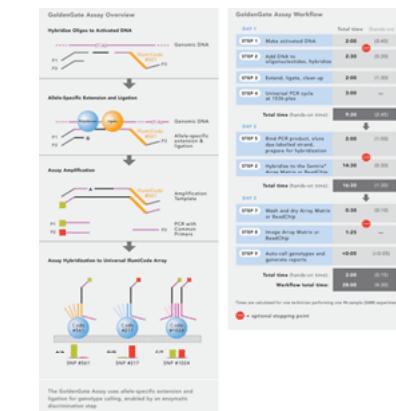
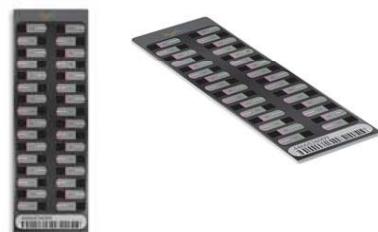


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DNA Test Panel Support



Genome Analyzer IIx Instrument

GoldenGate assay highlights

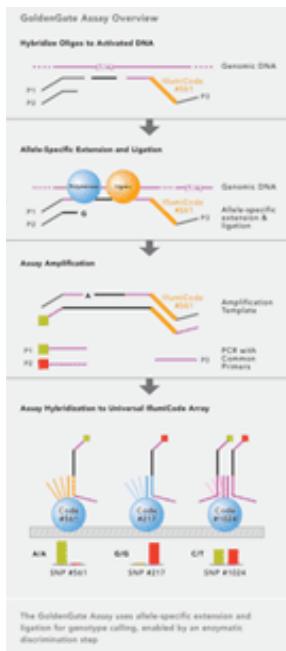
Proven technology: Used in genotyping centers worldwide

High-quality data: Average call rates > 99%

Scalable solution: 96, or from 384 to 3,072 SNPs per sample, 12 to 96 samples in parallel

Streamlined workflow: Manual or automated processing with multiple stopping points

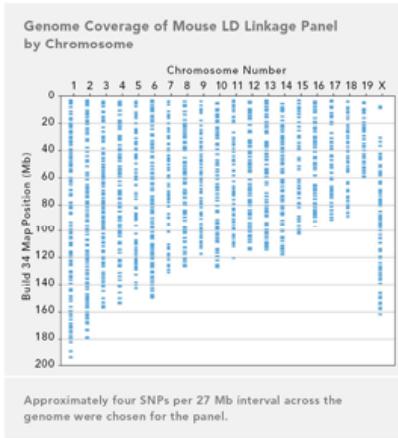
Flexible content: An expanding selection of standard panels or custom panels with the SNP loci of your choice



GoldenGate Assay Workflow

	Total Time (hands-on time)
DAY 1	
Step 1: Mix activated DNA	2:00 (0:45)
Step 2: Add DNA to oligolectinol, hybridize	2:00 (0:30)
Step 3: Extract, ligate, clean up	2:00 (1:30)
Step 4: Universal PCR cycle at 1536 place	3:00 —
Total Time (hands-on time)	9:30 (2:45)
DAY 2	
Step 5: Bind PCR product, elute and dilute, prepare for hybridization	2:00 (1:00)
Step 6: Hybridize to the Sentrix® Array Matrix or BeadChip	14:30 (0:30)
Total Time (hands-on time)	16:30 (1:30)
DAY 3	
Step 7: Wash and dry Array Matrix or BeadChip	0:30 (0:10)
Step 8: Image Array Matrix or BeadChip	1:25 —
Step 9: Auto-call genotypes and generate reports	>0:00 (>0:05)
Total Time (hands-on time)	1:45 (0:15)
Workflow total time:	21:00 (4:30)

Times are calculated for one technician performing one 96-sample (96x) experiment.
● = optional stopping point



www.geneticseducation.nhs.uk

Resource database

- Existing resources
- Resources developed by the Centre

The screenshot shows the homepage of the Supporting Genetics Education for Health website. At the top right is the NHS logo and the text "National Genetics Education and Development Centre". The main navigation menu includes Home, About Us, Learning Genetics, Teaching Genetics, and Genetics in Practice. Below the menu, there's a banner for the "Genetic Progress embedding scientific advances into Healthcare" initiative. A sidebar on the left lists "Latest Highlights" such as "Is my patient's condition inherited?", "A Medical History Drawing Tool for non-genetics health professionals", "The Genetics of Obesity quick reference sheets for the diabetic clinic", "Genetics clinical scenarios tutorials for medical students", "Medical School Genetics Teaching Leads Network", and "Genetics in Primary Care". The central content area features sections for "Resources", "Competences", "Centre Publications", and "Telling Stories". The "Resources" section includes a link to the "Core Family History Series". The "Competences" section discusses "Enhancing patient care by integrating genetic competences into clinical practice". The "Centre Publications" section highlights a study titled "Just Published! The experiences and preferences of people receiving genetic information from healthcare professionals". The "Telling Stories" section features a video thumbnail and text about "Planning a teaching session? and our Telling Stories, Understanding Real Life Genetics can support you." A "Welcome" section at the bottom left explains the website's purpose: "The NHS National Genetics Education and Development Centre is working with a range of groups throughout the UK to facilitate the integration of genetics education into all levels of education and training for all NHS health professionals. This website gives health professional educators and trainers access to genetics education resources. Those teaching genetics or learning about the impact of genetics knowledge on practice have access to searchable databases of resources and courses specific to their practice." At the bottom right are links for "Sitemap" and "Lorem ipsum dolor sit amet".

Searchable

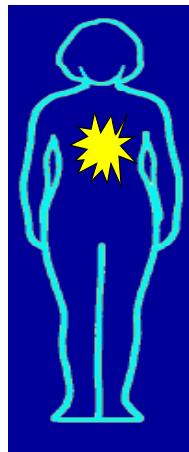
- Search all
- Linked to educational outcomes

The screenshot shows a search result page for "Teaching General Practice Trainees". The left sidebar lists categories for "Medical Practitioners" including "Medical Practitioners", "Learning and Training", "Assessing", "Teaching and Learning", "Nursing", "Pharmacy", and "Midwives". The main content area is titled "Teaching General Practice Trainees" and contains text about the resources available for GPs. A callout box highlights the third outcome: "3. Be able to identify patients with, or at risk of, a genetic condition". Below this, a bulleted list states: "• Be able to take a family history and construct and interpret a pedigree". To the right, there's a thumbnail for a resource titled "Core Family History Series" under the "Resources" category.

Evaluated

Most Cancers Arise From Somatic Mutations

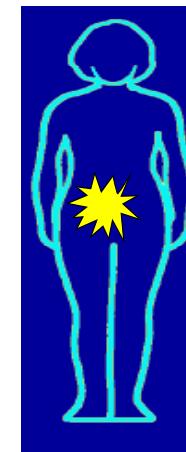
- Somatic mutation
 - Localised to a specific tissue



breast

or

bowel



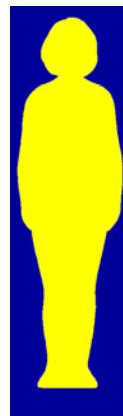
- Not in germline tissues
- Not inherited

5-10% of Cancers Arise From Germline Mutations

Parent

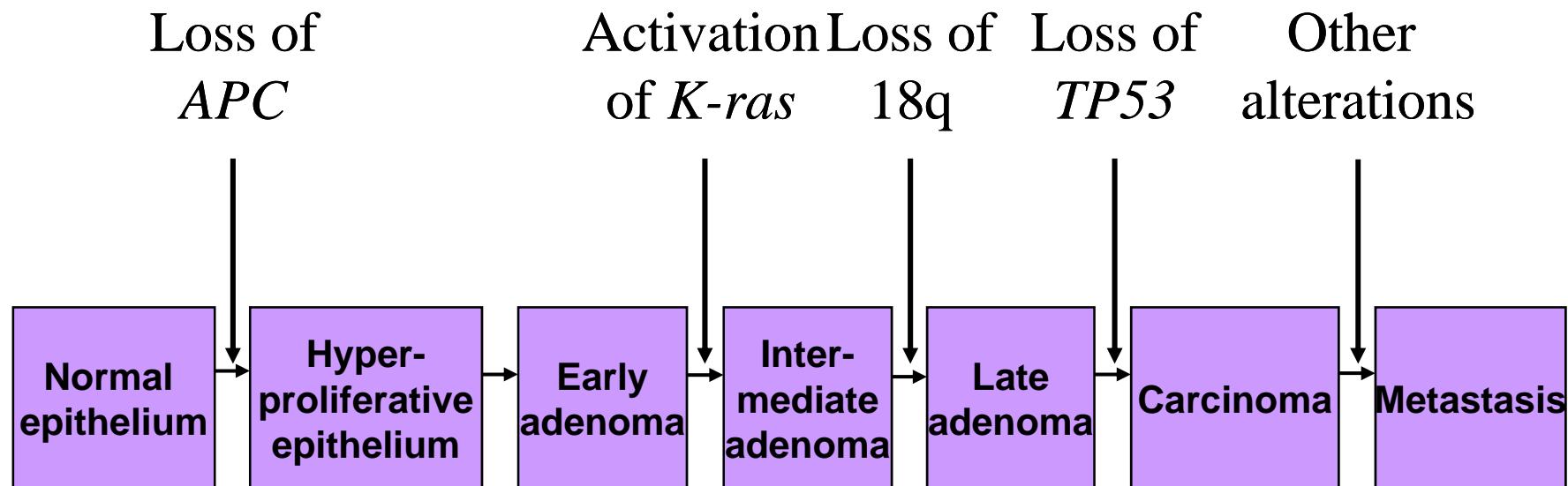


Child



- Germline mutation
 - In egg or sperm
 - May be passed on (inherited)
 - All cells in offspring carry the mutation

Multi-Step Carcinogenesis (eg, Colon Cancer)



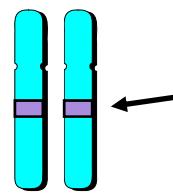
Adapted from Fearon ER. *Cell* 61:759, 1990

ASCO

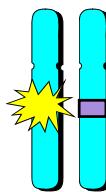
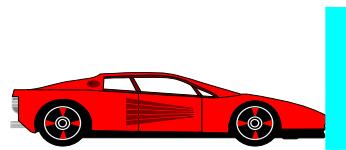
Genes Associated With Cancer

1. Tumor suppressor genes:

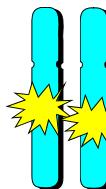
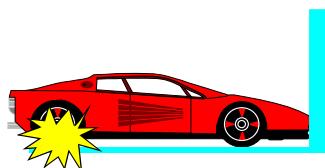
the cell's brakes for tumor growth



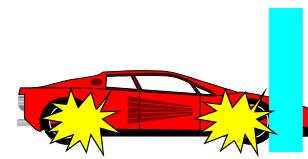
Normal genes
(prevent cancer)



1st mutation
(susceptible carrier)

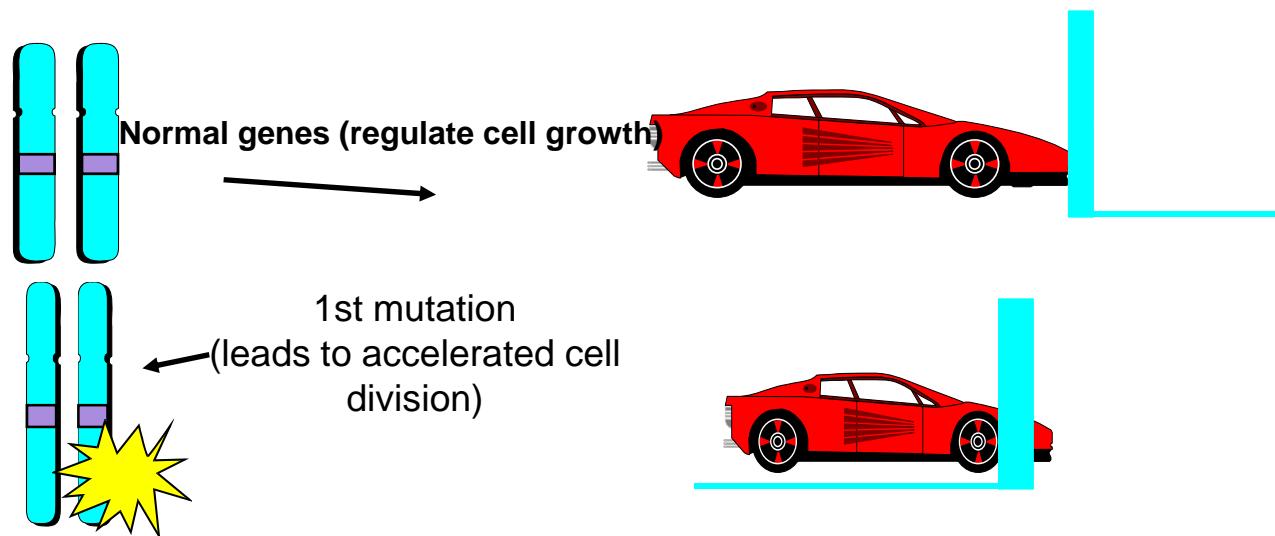


2nd mutation or loss
(leads to cancer)



Genes Associated With Cancer

2. Oncogenes: accelerate cell division

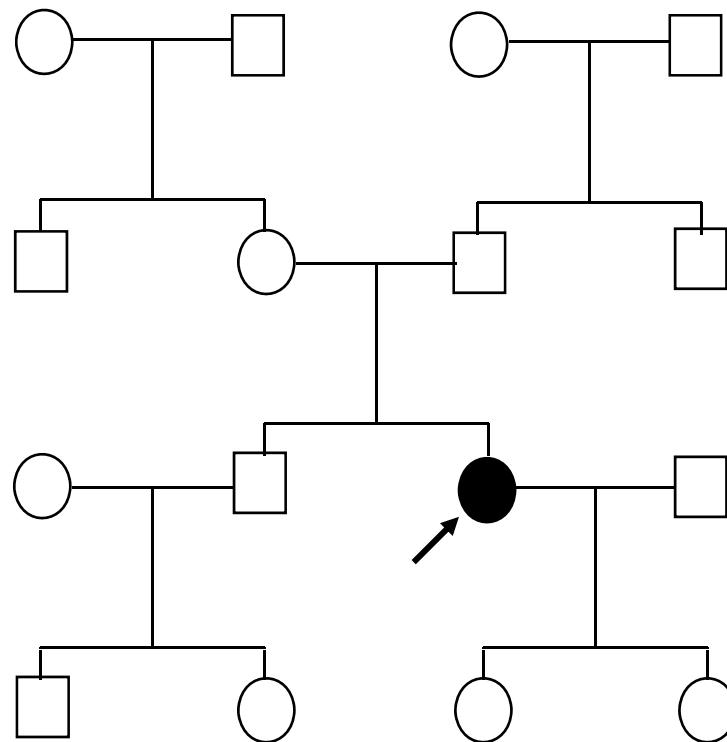


1 mutation sufficient for role in cancer development

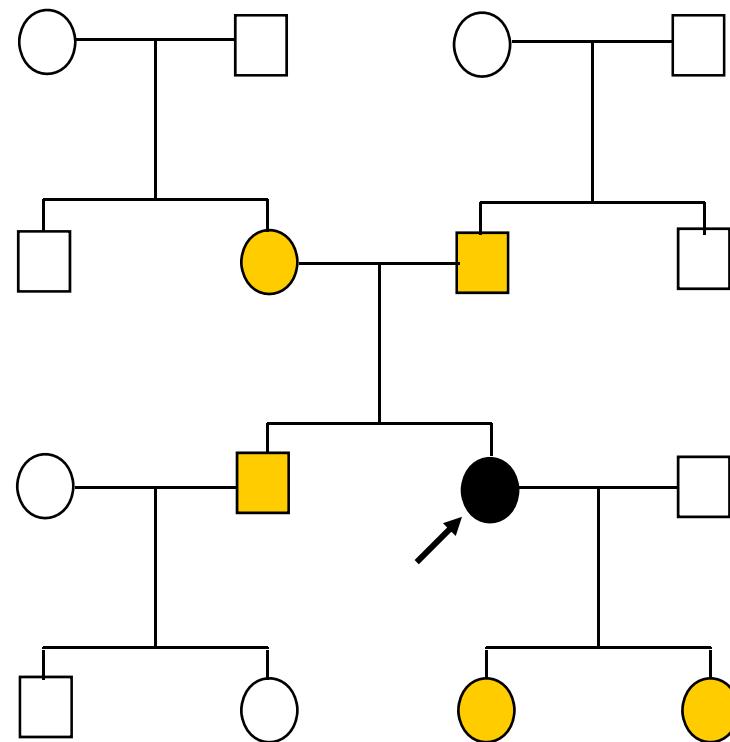
Examples of hereditary cancer genes

1p35	SDHA/B/C	Phaeochromocytoma/Paraganglioma
2p16	MSH2	HNPPCC
2p16	MSH6	HNPPCC
3p21	MLH1	HNPPCC
3p25	VHL	von Hippel-Lindau
5q21	APC	Colon polyposis
7p22	PMS2	HNPPCC
9p21	P16 (CDKN2)	Melanoma/pancreatic
10q22	PTEN	Cowdens syndrome
10q11	RET	MEN2
11q13	MEN1	MEN1
13q12	BRCA2	HBOC
13q14	RB1	Retinoblastoma
16q22.1	CDH1	Gastric cancer
17p13	TP53	Li-Fraumeni
17q21	BRCA1	HBOC

Half of the population have had a 1st or 2nd degree relative diagnosed with cancer

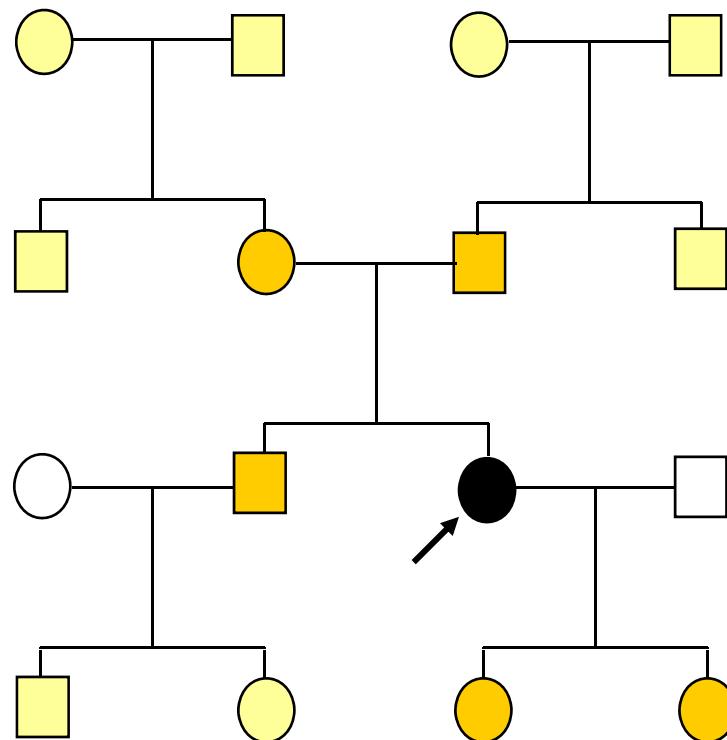


Half of the population have had a 1st or 2nd degree relative diagnosed with cancer



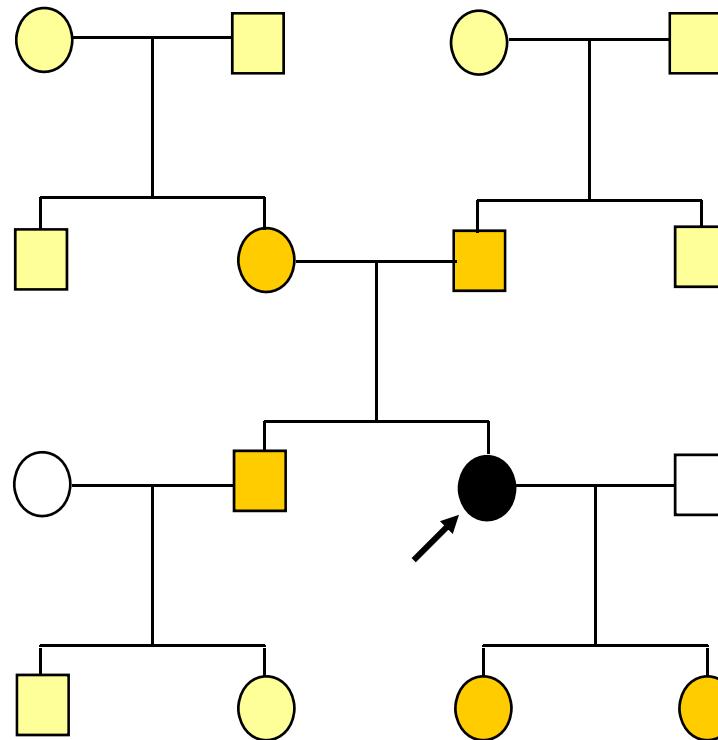
**1st degree
(parents, siblings,
children)**

Half of the population have had a 1st or 2nd degree relative diagnosed with cancer



**2nd degree
(grandparents,
aunts, uncles,
nieces, nephews)**

Half of the population have had a 1st or 2nd degree relative diagnosed with cancer



**2nd degree
(grandparents,
aunts, uncles,
nieces, nephews)**

Only 5% - 10% will have an inherited genetic factor

Gene mutations and inherited cancer

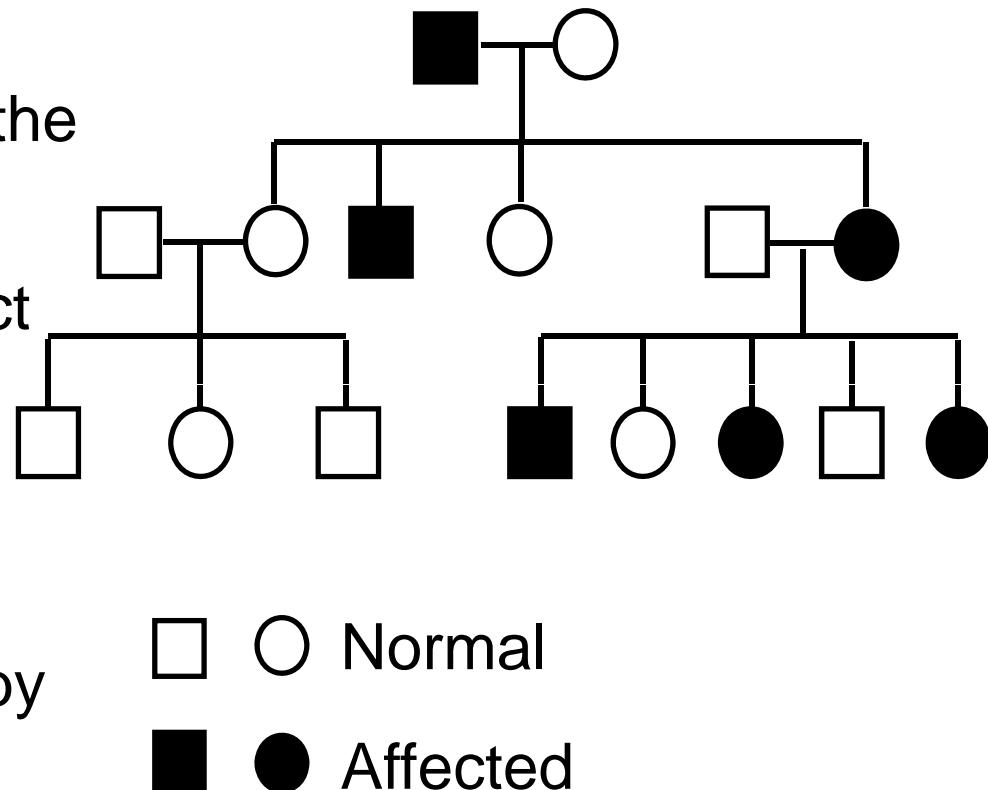
How are cancer
predispositions inherited?

Heredity Breast Cancer (high risk)

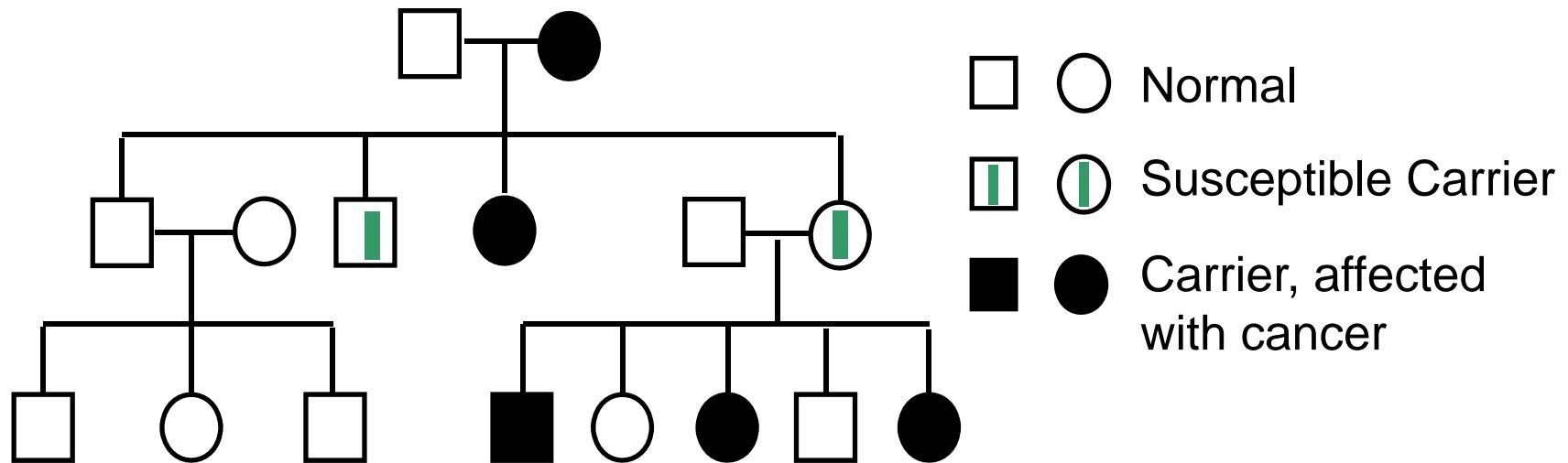
- ~5% of all Breast Cancers
- Monogenetic disorder
- Autosomal dominant inheritance
- High penetrance
- Early onset
- Cancer syndrome (other cancers also)
- BRCA1+2 only known genes of major importance

Autosomal Dominant Inheritance

- Each child has 50% chance of inheriting the mutation
- Equally likely to affect males and females
- No “skipped generations”
- Equally transmitted by men and women

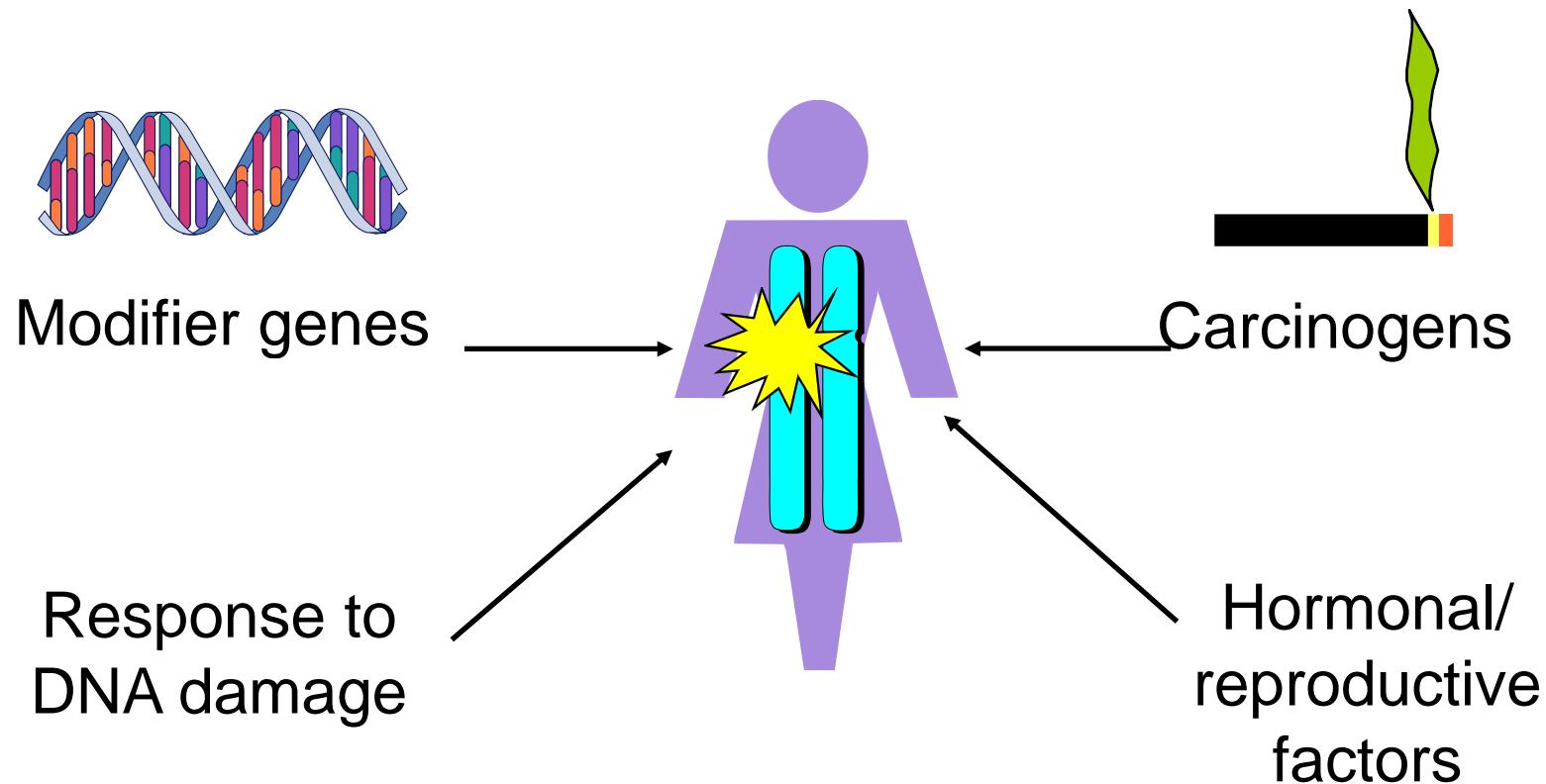


Penetrance



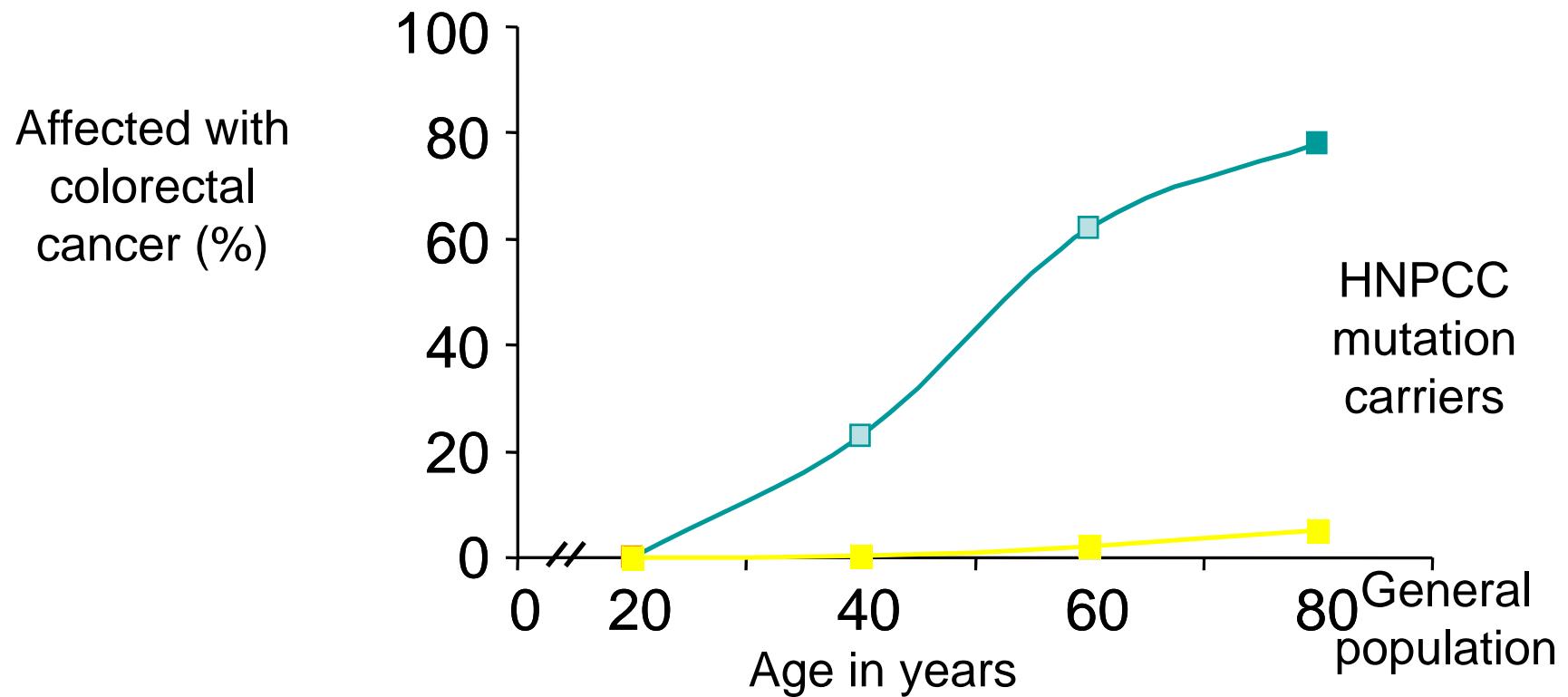
- May appear to “skip” generations
- Individuals inherit altered cancer susceptibility gene - not cancer

Factors Affecting Penetrance



Not everyone with an altered gene develops cancer

Age-Specific Penetrance

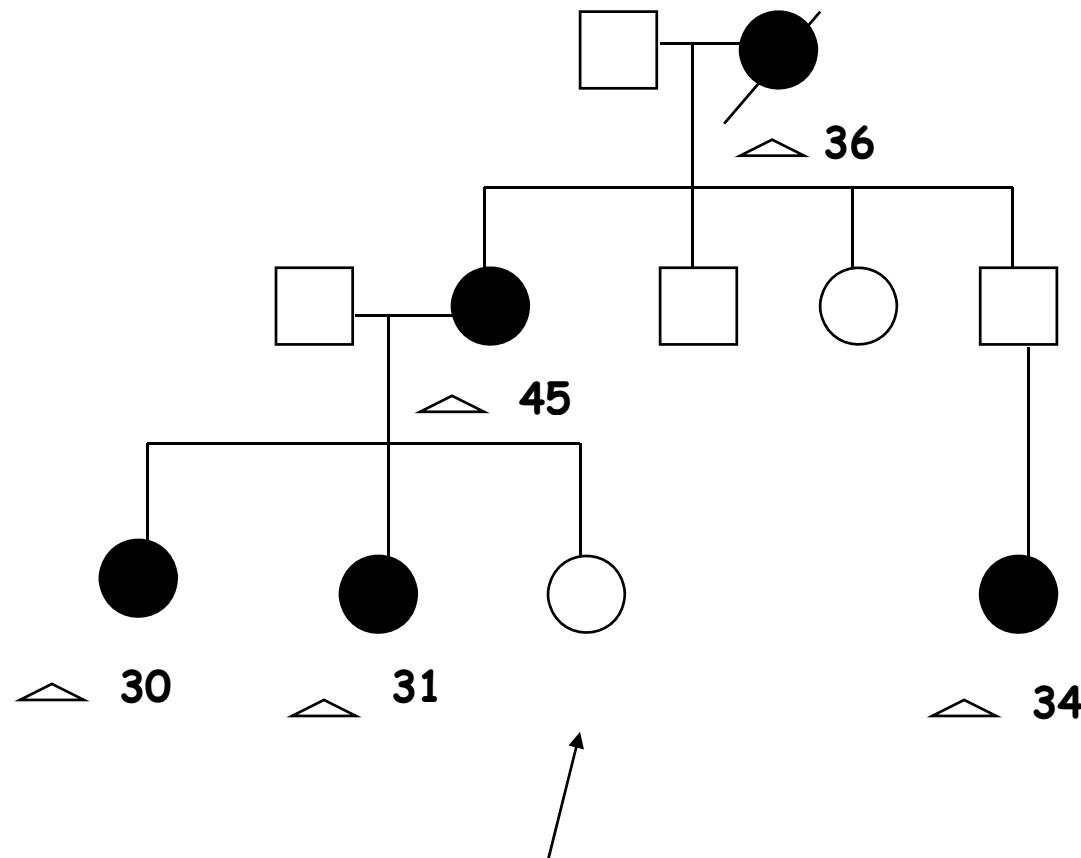


Modified from Aarnio M et al. *Int J Cancer* 64:430, 1995

High Risk Indicators

- Multiple family members with tumours at same site
- Early age of onset
- History of individuals with multiple primary tumours
- Recognised associations:
 - Breast/ovary
 - Bowel/Endometrium
 - etc.

Breast Cancer



NICE - familial breast cancer

Mammographic surveillance

High risk:

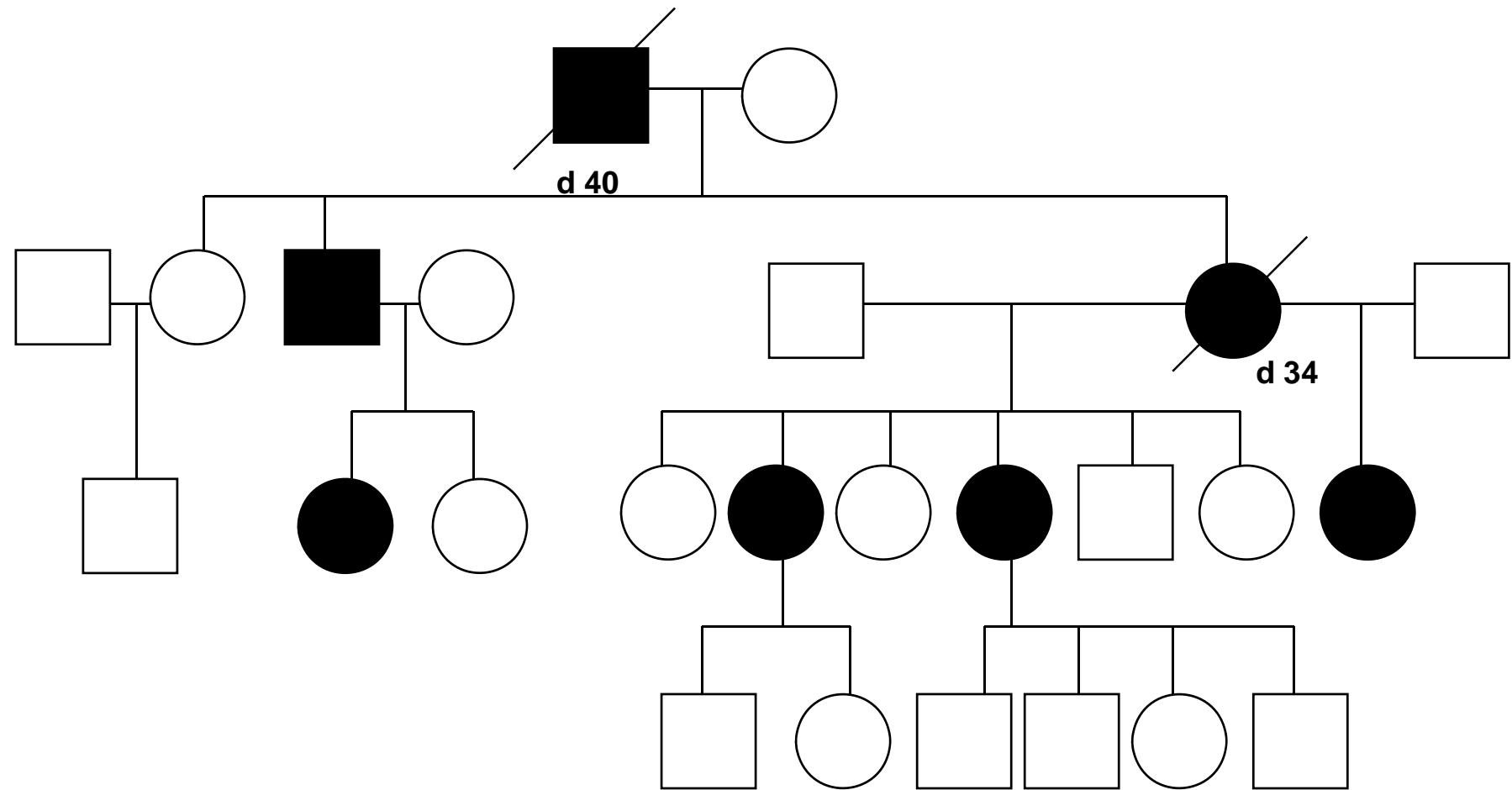
30-40 *individualised strategies*

- Mammography or MRI?

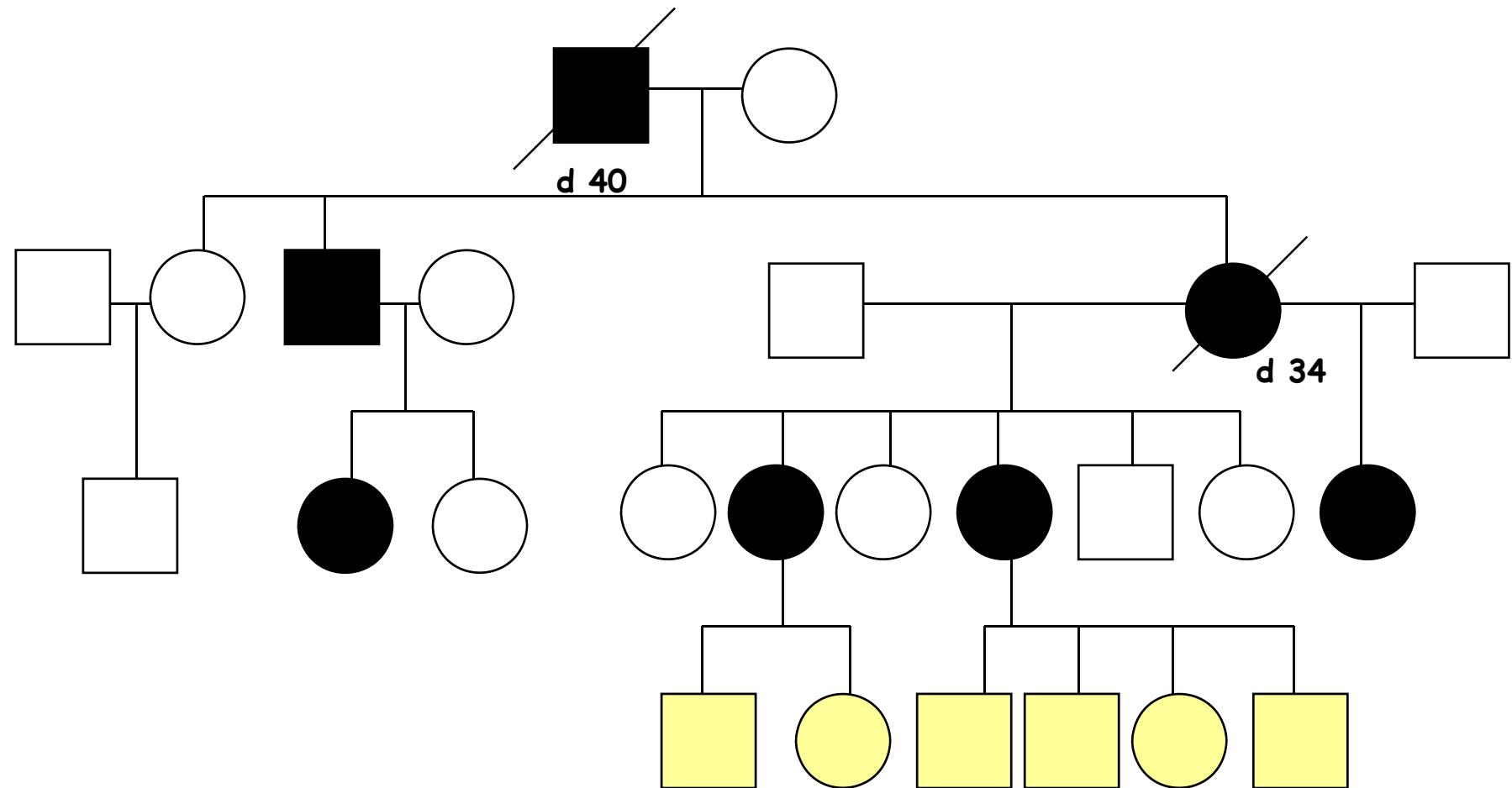
40-50 annual

50+ *individualised strategies*

FAP



FAP



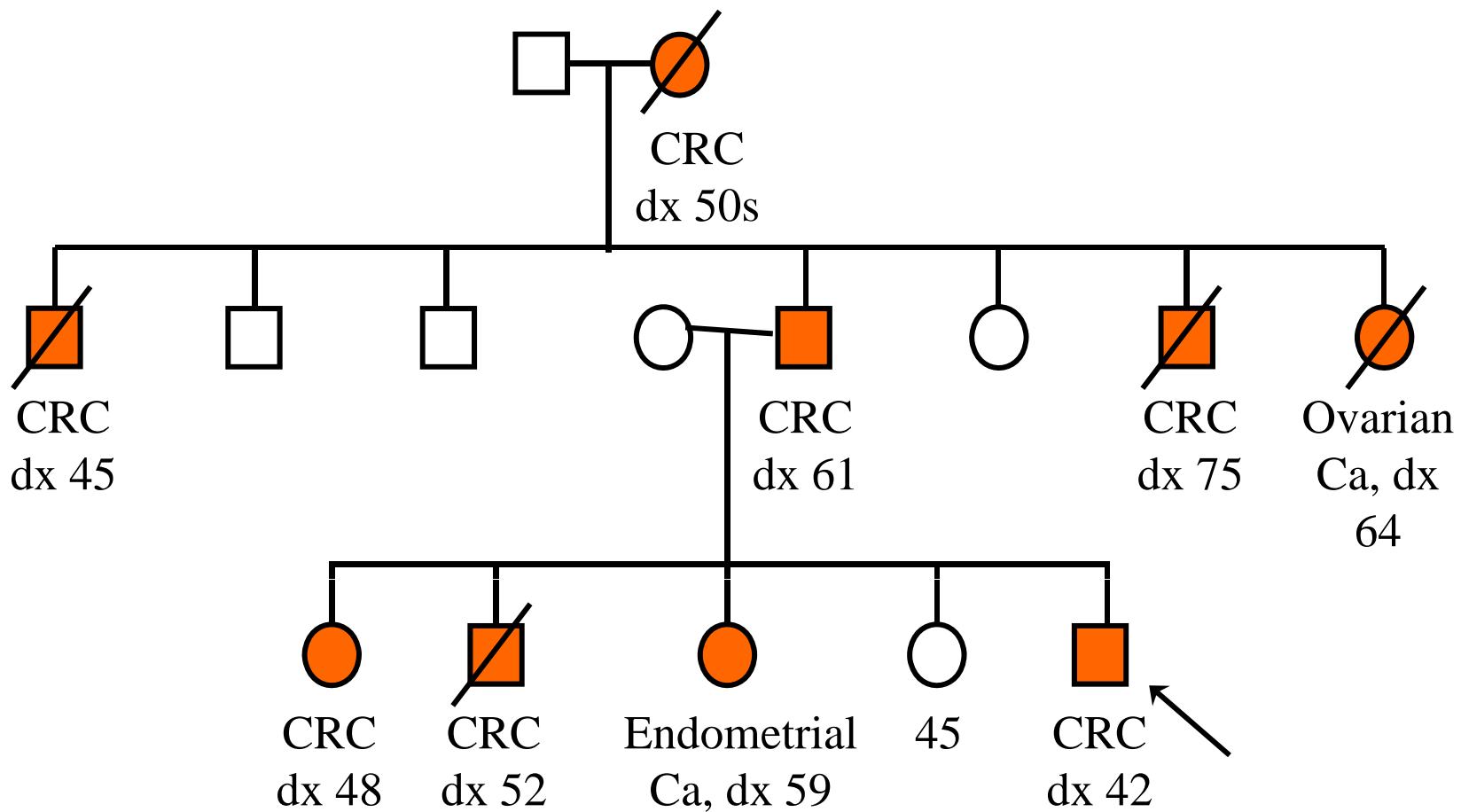
Genetics of FAP

- Caused by mutations in APC gene (found on chromosome 5)
- ~30% occur as the result of new mutations
- Correlation between position of mutation &:
 - Severity of effect
 - Presence of CHRPEs and desmoids

FAP: Key Points

- CRC risk is 100% in untreated FAP patients
- Genetic testing identifies most APC mutation carriers
- Endoscopic surveillance and prophylactic colectomy can improve survival in at-risk patients
- Non-carriers can be spared anxiety and the need for increased surveillance

Family History is the key to diagnosing HNPCC



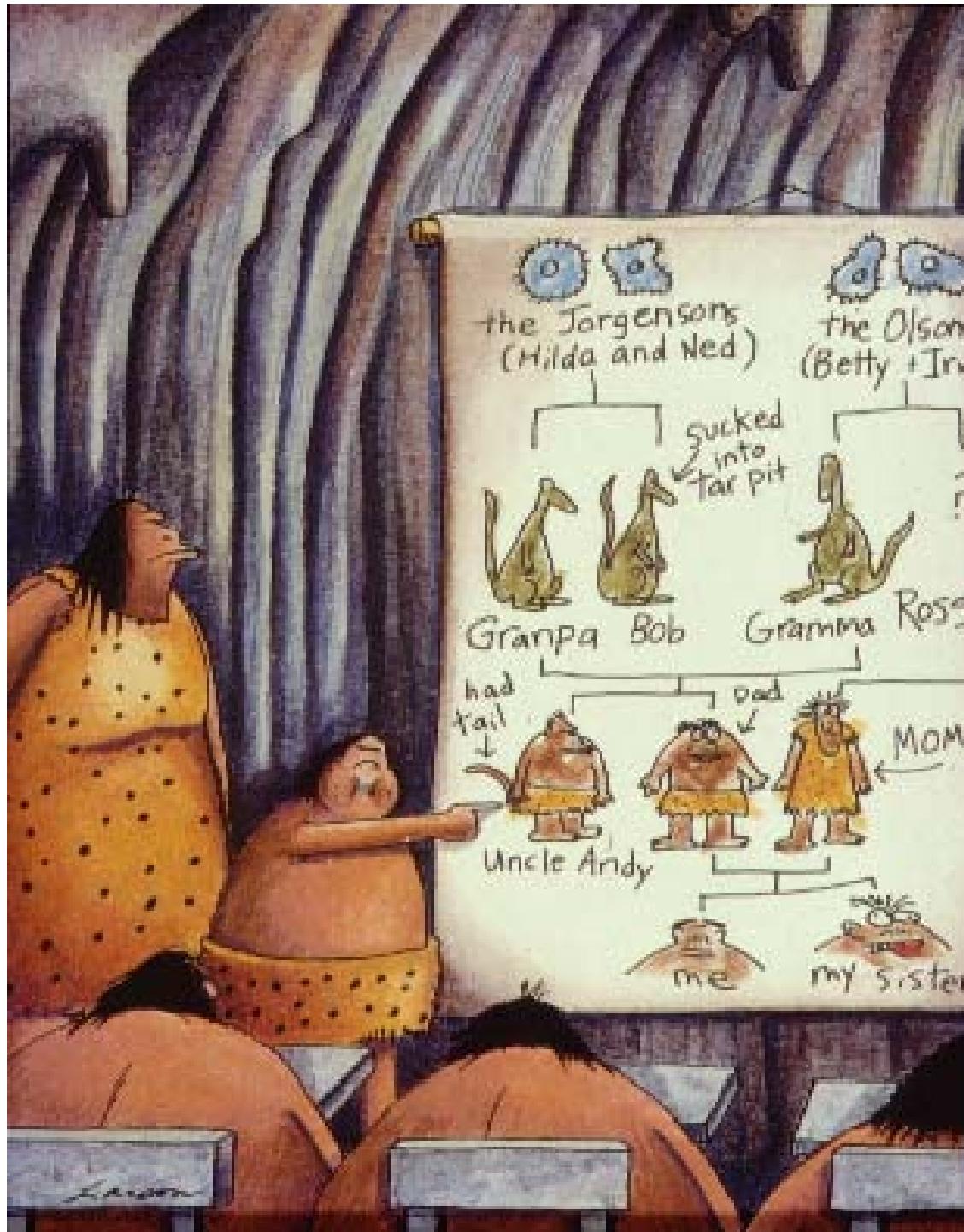
Genetic Features of HNPCC

- Autosomal dominant inheritance
- Penetrance ~80%
- Genes belong to DNA mismatch repair family
- A number of genes involved
(MLH1, MSH2, MSH6, PMS1, PMS2)

Amsterdam Criteria

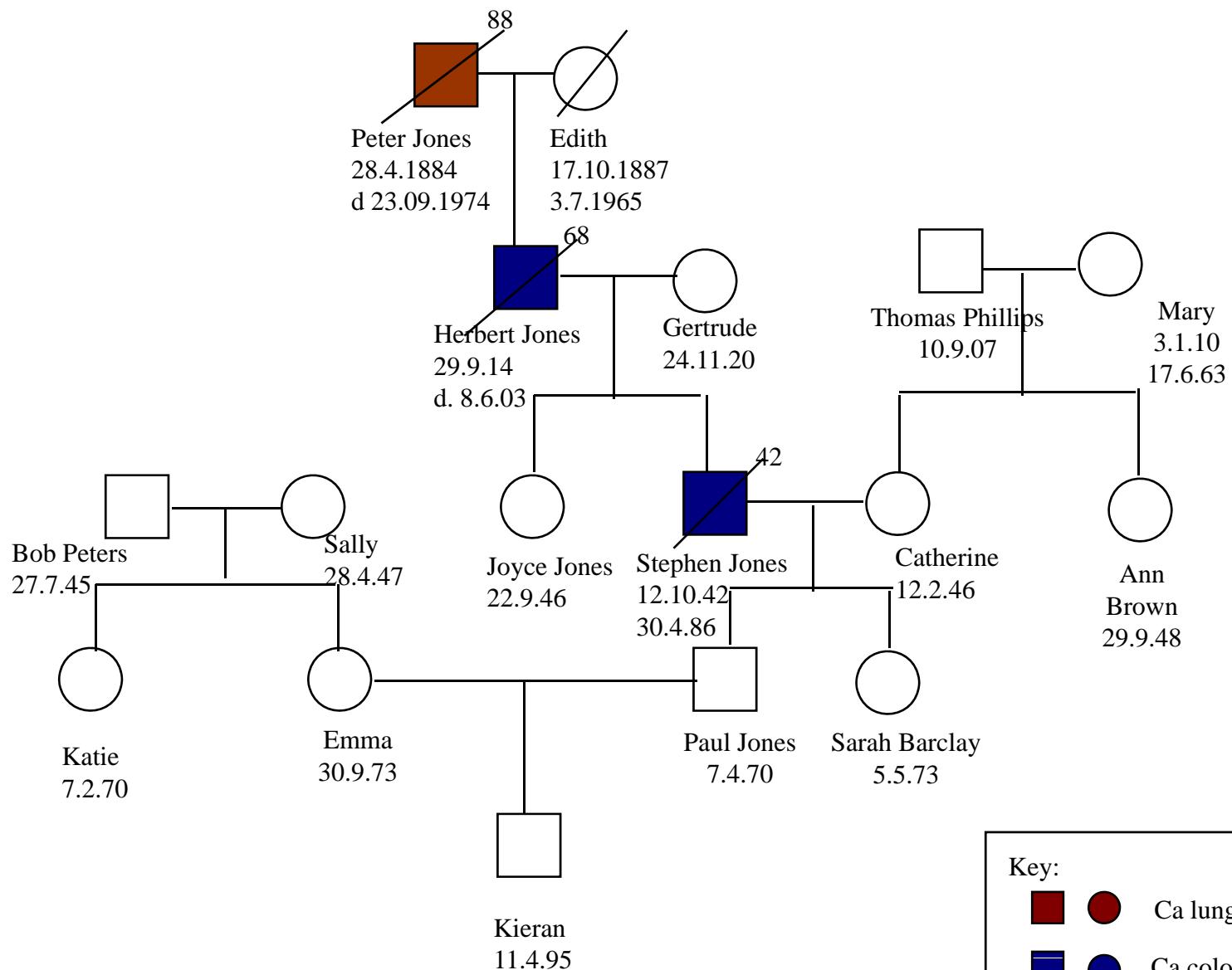
- 3 or more relatives with CRC
 - One case a 1^o degree relative of the others
 - Two or more generations
 - One CRC by age 50
 - FAP excluded
-
- Modified Amsterdam criteria: An endometrial cancer can be substituted for one of the CRC

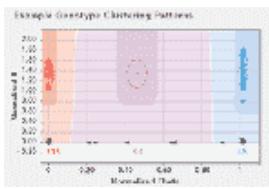
Taking a Pedigree



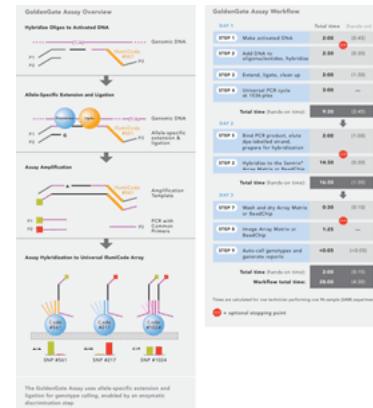
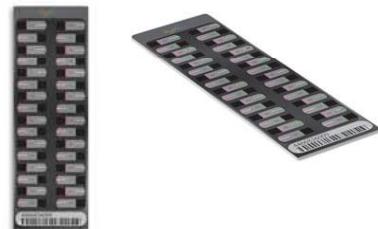
Drawing up the family tree gives information about the relatives and also:

- helps establish the family agenda and dynamics
- may reveal individuals interpretation and beliefs about what is happening in the family
- Has the potential to raise issues of paternity





DNA Test Panel Support



Genome Analyzer IIx Instrument

GoldenGate assay highlights

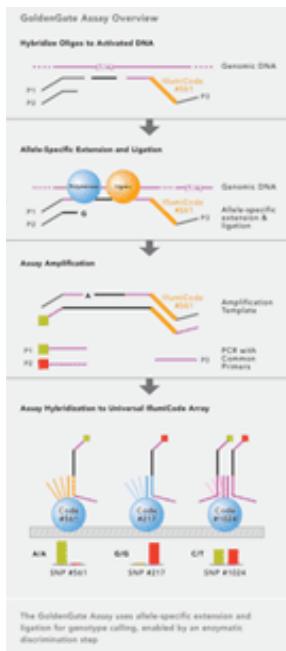
Proven technology: Used in genotyping centers worldwide

High-quality data: Average call rates > 99%

Scalable solution: 96, or from 384 to 3,072 SNPs per sample, 12 to 96 samples in parallel

Streamlined workflow: Manual or automated processing with multiple stopping points

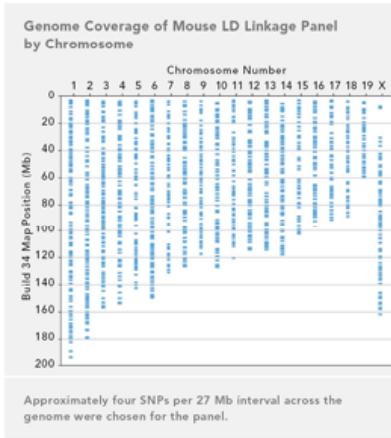
Flexible content: An expanding selection of standard panels or custom panels with the SNP loci of your choice



GoldenGate Assay Workflow

	Total Time (hands-on time)
DAY 1	
Step 1: Mix activated DNA	2:00 (0:45)
Step 2: Add DNA to oligolectinol, hybridize	2:00 (0:30)
Step 3: Extract, ligate, clean up	2:00 (1:30)
Step 4: Universal PCR cycle at 1536 place	3:00 —
Total Time (hands-on time)	9:30 (2:45)
DAY 2	
Step 5: Bind PCR product, elute and dilute, prepare for hybridization	2:00 (1:00)
Step 6: Hybridize to the Sentrix® Array Matrix or BeadChip	14:30 (0:30)
Total Time (hands-on time)	16:30 (1:30)
DAY 3	
Step 7: Wash and dry Array Matrix or BeadChip	0:30 (0:10)
Step 8: Image Array Matrix or BeadChip	1:25 —
Step 9: Auto-call genotypes and generate reports	>0:00 (>0:05)
Total Time (hands-on time)	1:45 (0:15)
Workflow total time:	21:00 (4:30)

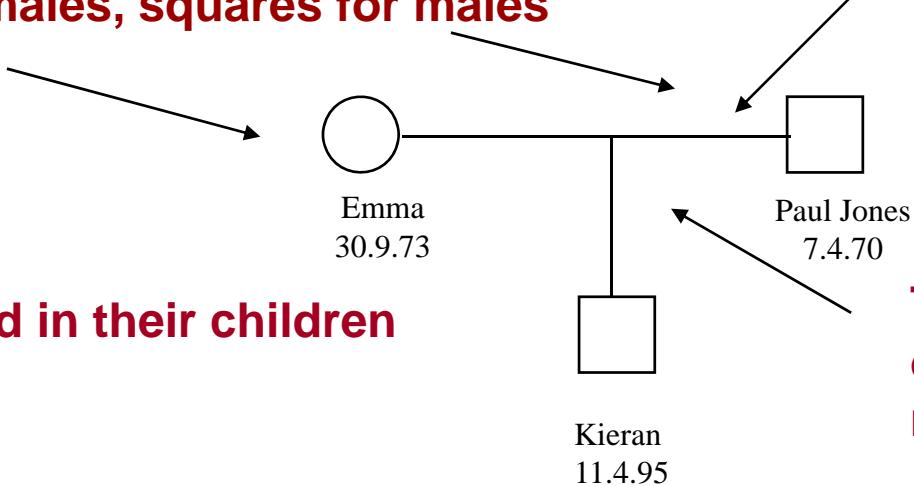
Times are calculated for one technician performing one 96-sample (96x) experiment.
● = optional stopping point



Start with the couple being seen

Use clear symbols: circles for females, squares for males

Add in their children

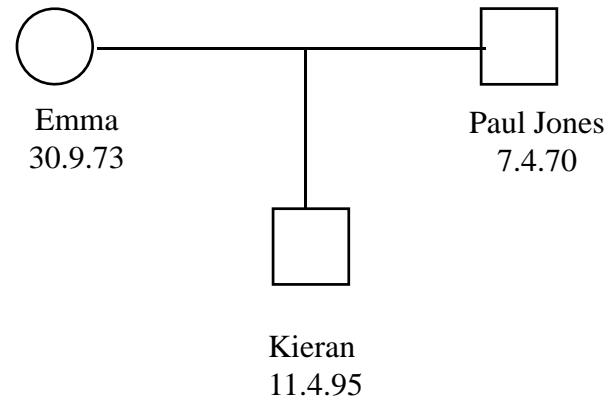


The horizontal line denotes a relationship (males usually on the left, females on right)

The vertical line denotes offspring of the relationship

“Have you had any children with other partners?”

Record names, dates of birth

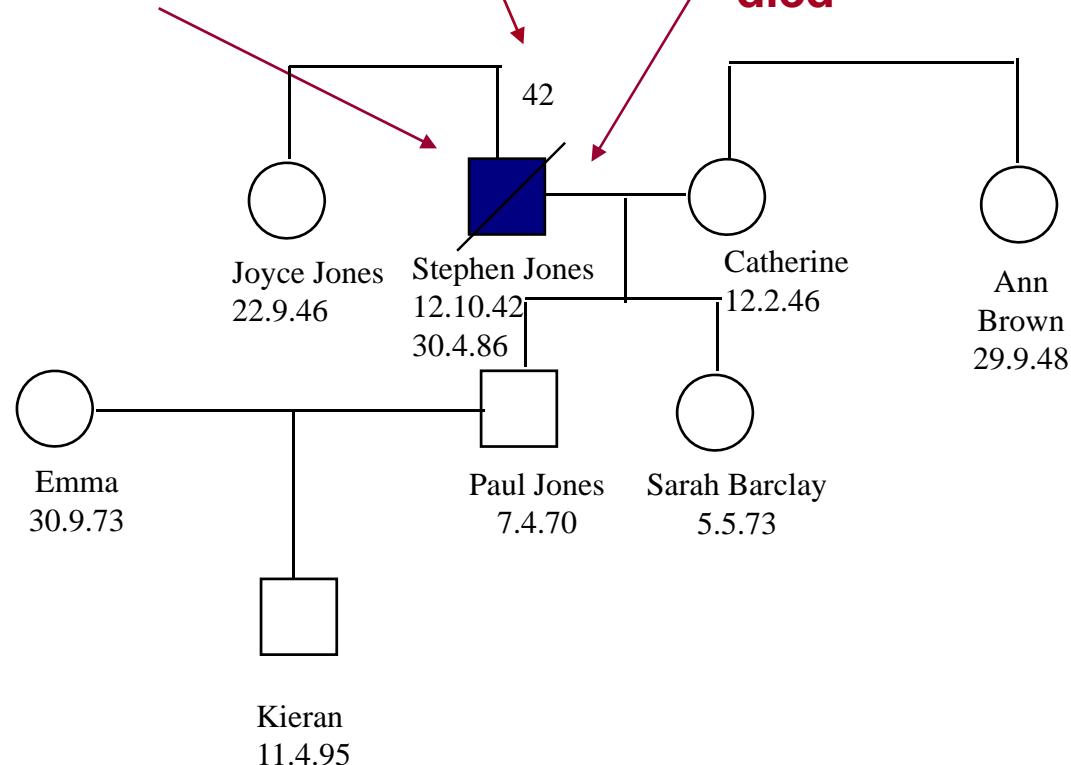


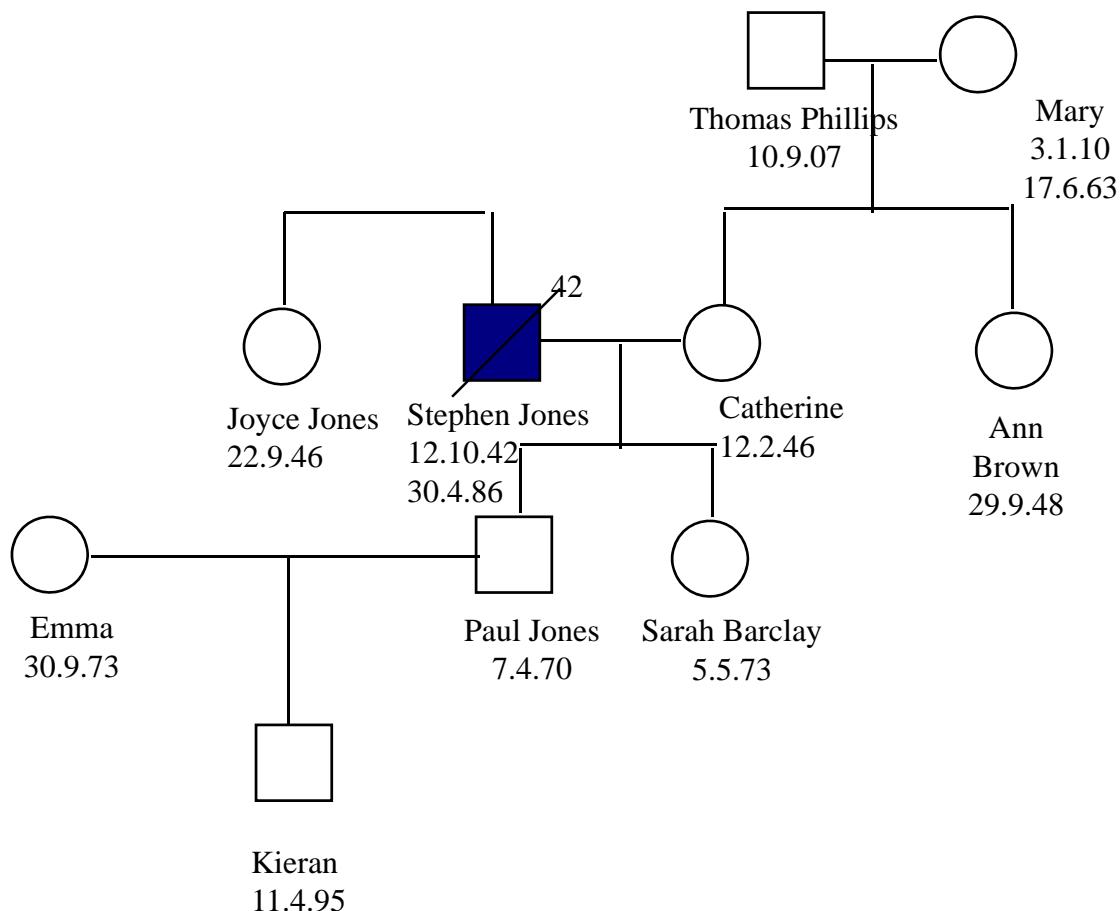
**Choose one parent and ask about:
brothers and sister and their children
parents and
grandparents
Make sure you ask about ethnicity**

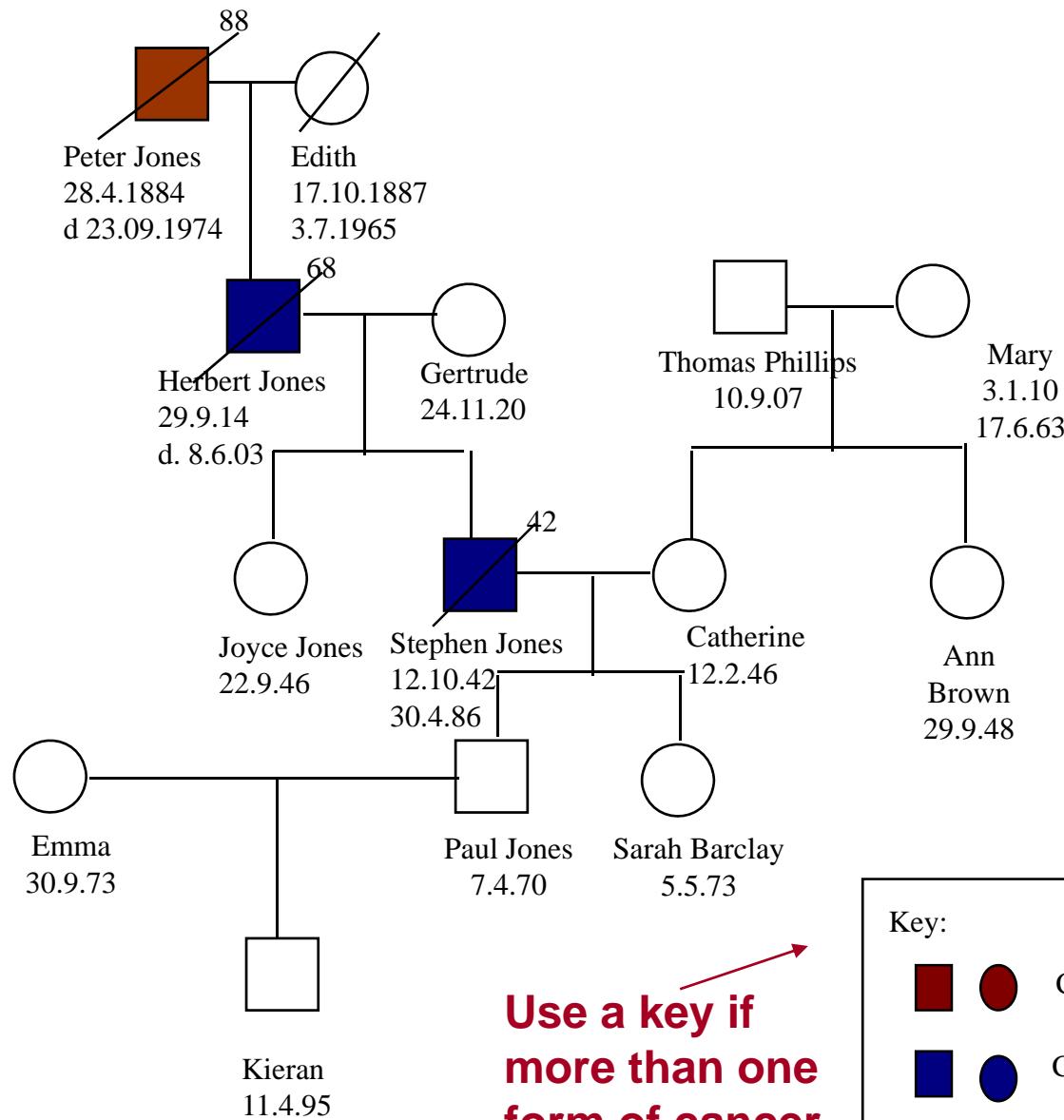
Colour in the symbol if the person is affected

Add the age at which diagnosis was made

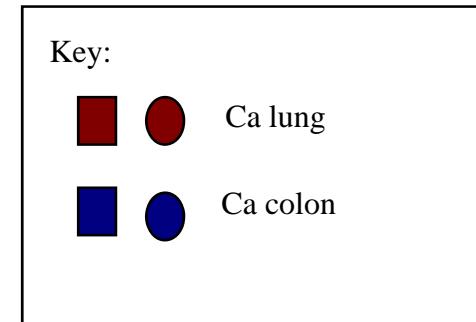
Put a sloping line through the symbol (from the bottom left hand corner) if the person has died

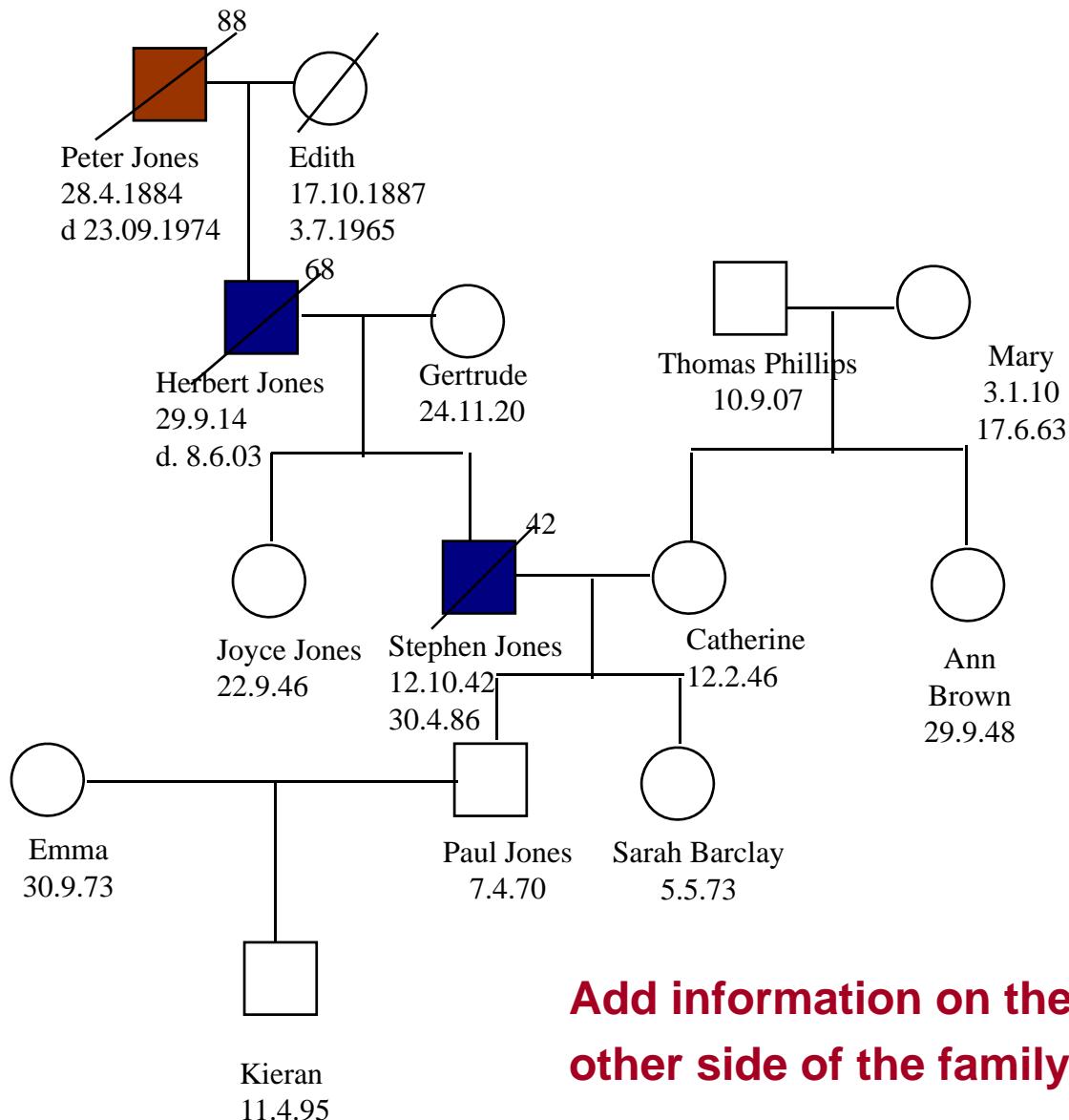


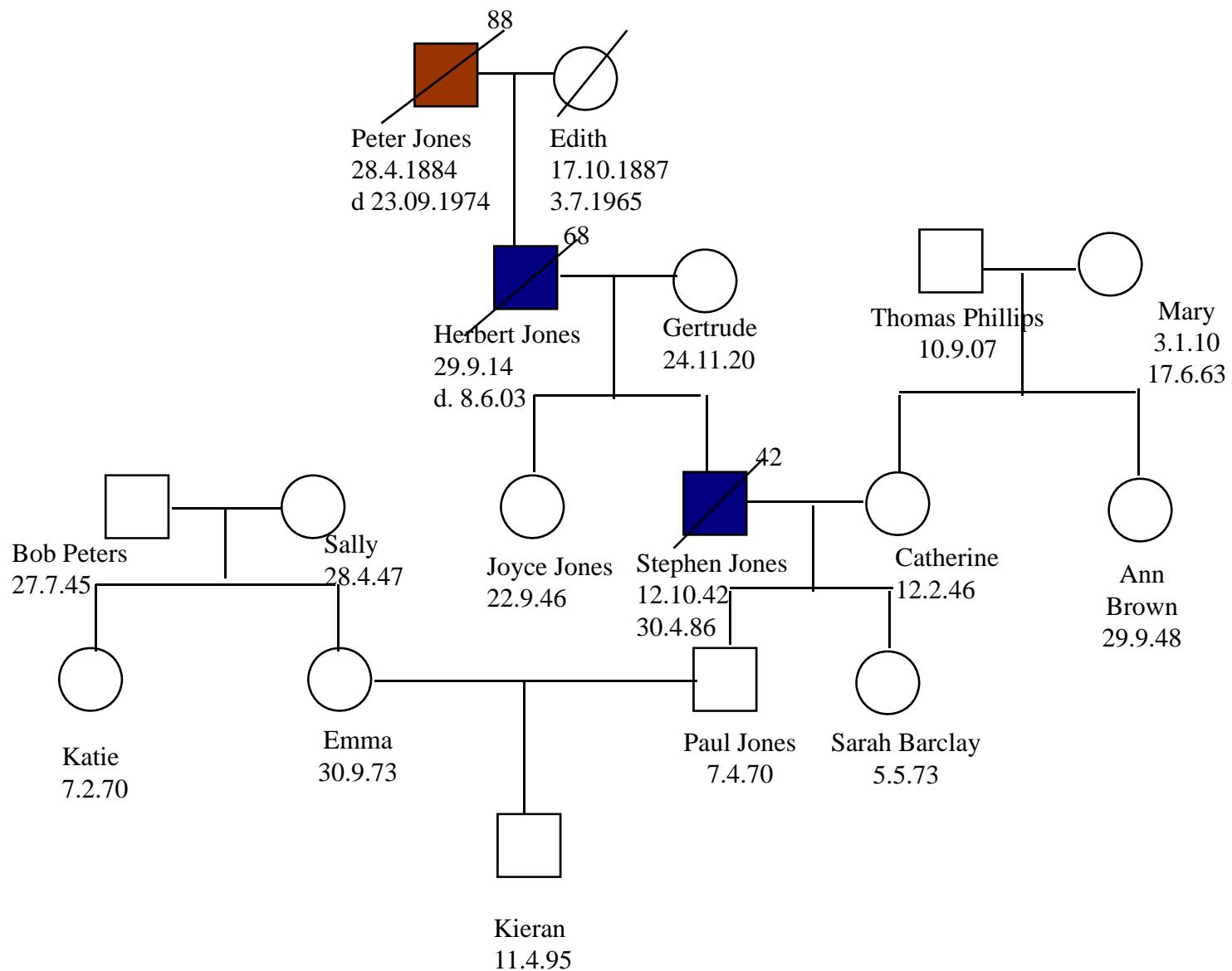




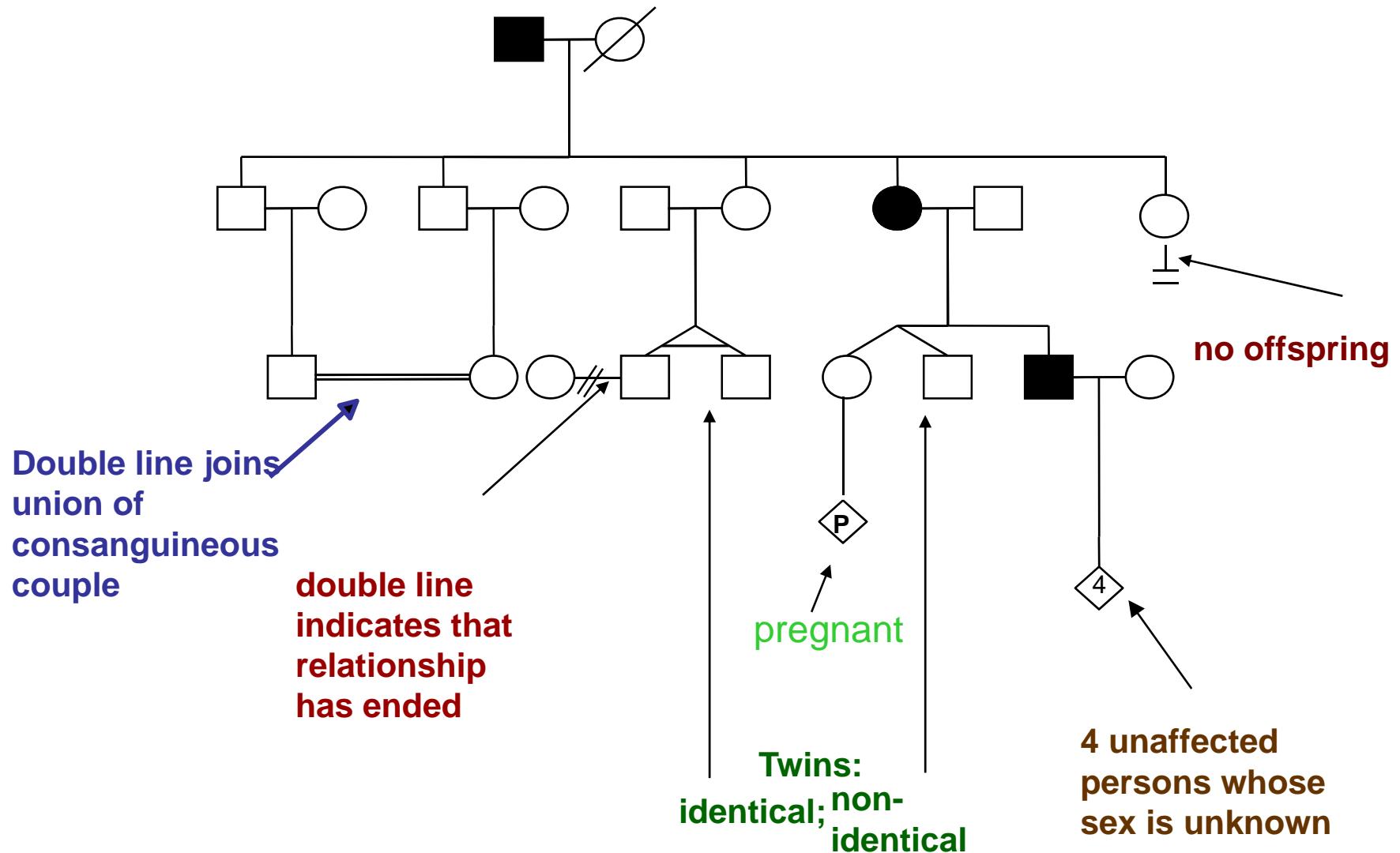
**Use a key if
more than one
form of cancer**



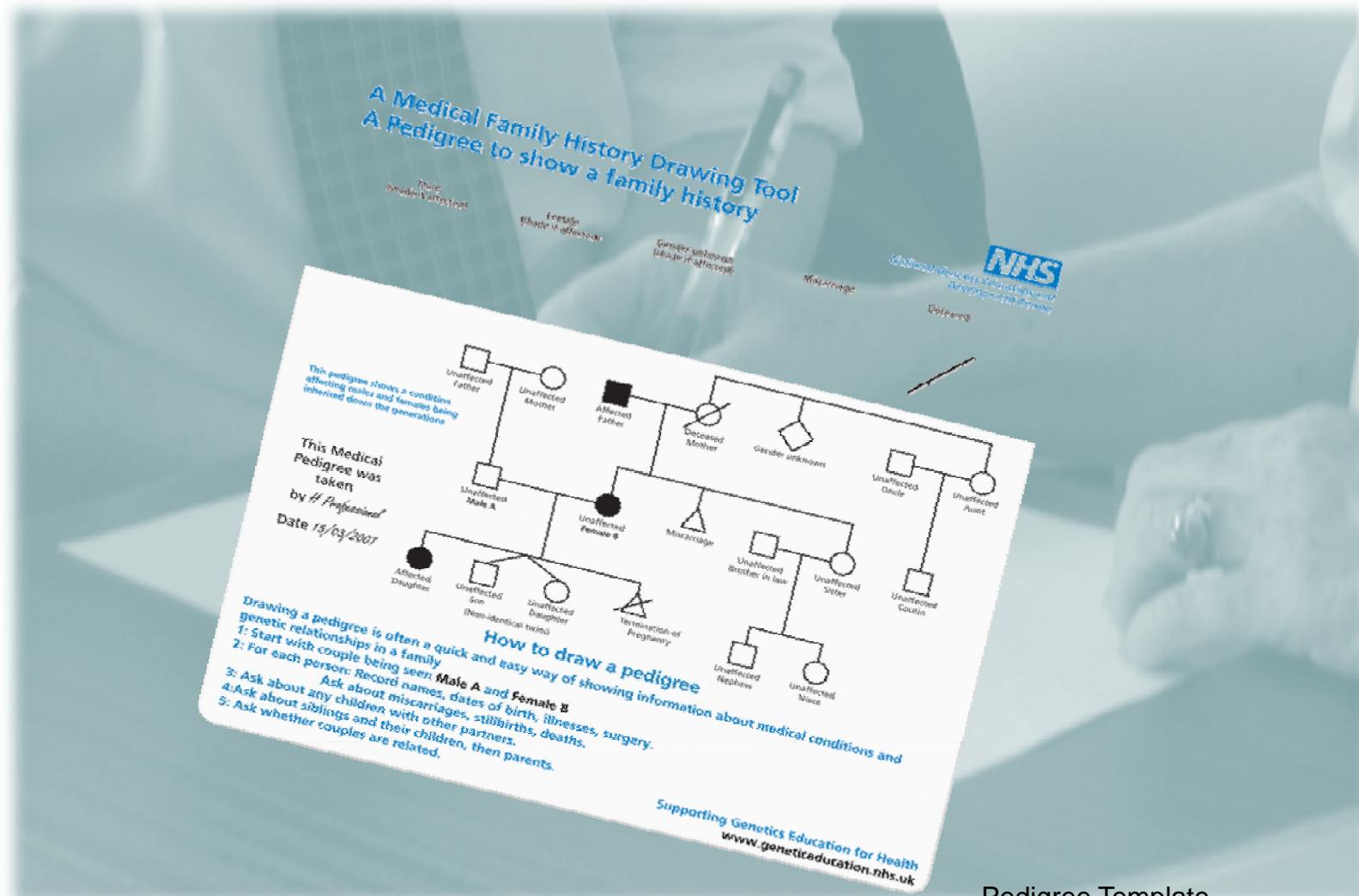




Other pedigree symbols



Drawing a pedigree



Pedigree Template-
One of the resources available from
the NHS National Genetics
Education and Development Centre

www.geneticseducation.nhs.uk

Resource database

- Existing resources
- Resources developed by the Centre

The screenshot shows the homepage of the Supporting Genetics Education for Health website. At the top right is the NHS logo and the text "National Genetics Education and Development Centre". The main navigation menu includes Home, About Us, Learning Genetics, Teaching Genetics, and Genetics in Practice. Below the menu, there's a banner for the "Genetic Progress embedding scientific advances into Healthcare" initiative. A sidebar on the left lists "Latest Highlights" such as "Is my patient's condition inherited?", "A Medical History Drawing Tool for non-genetics health professionals", "The Genetics of Obesity quick reference sheets for the diabetic clinic", "Genetics clinical scenarios tutorials for medical students", "Medical School Genetics Teaching Leads Network", and "Genetics in Primary Care". The central content area features sections for "Resources", "Competences", "Centre Publications", and "Telling Stories". The "Resources" section includes a link to the "Core Family History Series". The "Competences" section discusses "Enhancing patient care by integrating genetic competences into clinical practice". The "Centre Publications" section highlights a study titled "Just Published! The experiences and preferences of people receiving genetic information from healthcare professionals". The "Telling Stories" section features a video thumbnail and text about "Planning a teaching session? and our Telling Stories, Understanding Real Life Genetics can support you." A "Welcome" section at the bottom left explains the website's purpose: "The NHS National Genetics Education and Development Centre is working with a range of groups throughout the UK to facilitate the integration of genetics education into all levels of education and training for all NHS health professionals. This website gives health professional educators and trainers access to genetics education resources. Those teaching genetics or learning about the impact of genetics knowledge on practice have access to searchable databases of resources and courses specific to their practice." At the bottom right are links for "Sitemap" and "Lorem ipsum dolor sit amet".

Searchable

- Search all
- Linked to educational outcomes

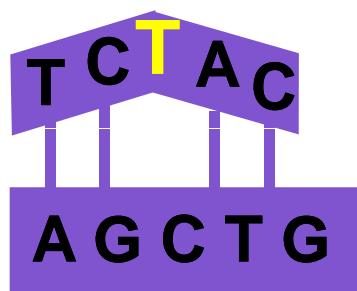
The screenshot shows a search result page for "Teaching General Practice Trainees". The left sidebar lists categories for "Medical Practitioners" including "Medical Practitioners", "Learning and Training", "Assessing", "Teaching and Learning", "Nursing Proficiency", "Pharmacy", and "Sisters". The main content area is titled "Teaching General Practice Trainees" and contains text about the resources available for GPs. A callout box highlights the third outcome: "3. Be able to identify patients with, or at risk of, a genetic condition". Below this, a bulleted list states: "• Be able to take a family history and construct and interpret a pedigree". To the right, there's a thumbnail for a resource titled "Core Family History Series" under the "Resources" category.

Evaluated

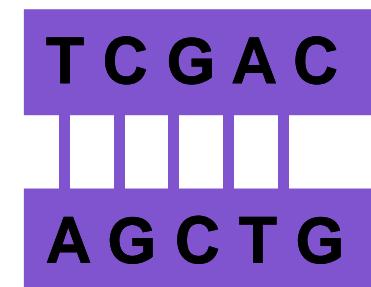
Genes Associated With Cancer

3. DNA damage-response genes: the repair mechanics for DNA

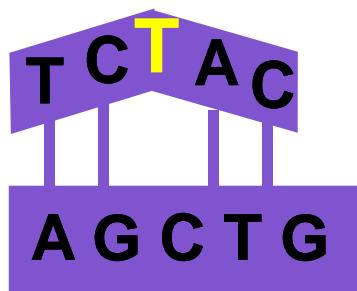
Base pair
mismatch



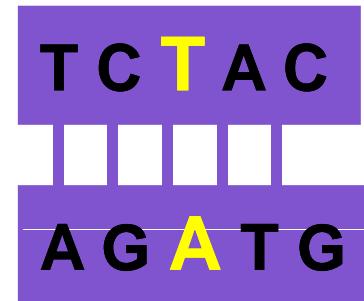
Normal
DNA repair



DNA damage-response genes:



Base pair
mismatch



Mutation
introduced by
unrepaired
DNA