cancer is fundamentally a genetic disease II

Dr. Bilal Azab

Familial Breast Cancer due to Mutations in BRCA1 and BRCA2

- In the general female population. An individual woman has a 1-in-8 (12%) chance of developing breast cancer over an 80-year lifespan
- (≈3% to 5%) of breast cancer cases appear to be due to a highly penetrant **dominantly inherited mendelian predisposition** that increases the risk for female breast cancer fourfold to sevenfold over the 12% lifetime risk observed in the general female population.
- In these families, one often sees features characteristic of hereditary (as opposed to sporadic) cancer: multiple affected individuals in a family, earlier age at onset, frequent multifocal (appears in more than one region in the breast) , bilateral disease or second independent primary breast tumor, and second primary cancers in other tissues such as ovary and prostate.

- Breast cancer can be **hereditary or non-hereditary**. ***Hereditary*** breast cancer results from germline mutations and tends to run in families. In contrast, ***non-hereditary*** (sporadic) breast cancer results from somatic mutations that occur spontaneously in breast tissue and does not typically run in families.

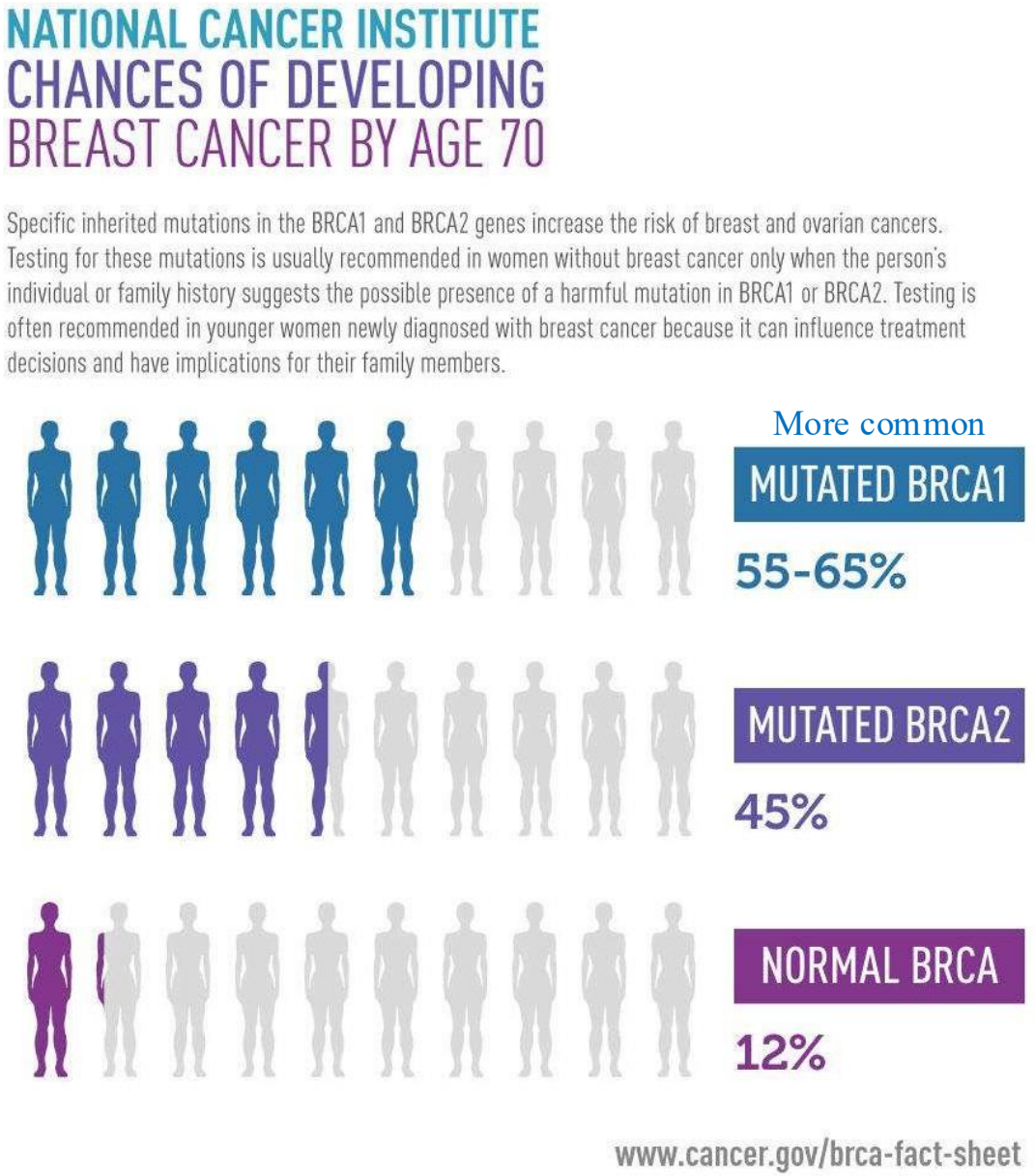
- BRCA1 and BRCA2 (tumor suppressor genes) are responsible for the majority of all hereditary breast cancers

- Together, these two TSGs account for approximately one **half** and one **third**, respectively, of autosomal dominant familial breast cancer.

- (NCBI: Breast **cancer risks** are at
  - ❑ 40–87% for **BRCA1 mutation carriers** (higher risk)
  - ❑ 18–88% for **BRCA2 mutation carriers**.

- For ovarian **cancer**, the **risk** estimates are in the range of:
  - ❑ 22–65% for **BRCA1** (higher risk)
  - ❑ 10–35% for **BRCA2**.

- Mutations in BRCA1 and BRCA2 are also associated with a significant increase in the risk for ovarian and fallopian duct cancer in female heterozygotes.



- Breast cancer is the most common malignancy in individuals with a germline BRCA1 or BRCA2 pathogenic (oncogenic) variant with a lifetime risk ranging from 46% to 87%.

✓ If a female has a germline disease-causing variant, she is born with the first hit already present. According to the ***two-hit theory***, there is a high probability that a second hit will occur over time. Once the second hit occurs, the disease manifests.

- *That is why many females with pathogenic BRCA1 or BRCA2 variants choose to undergo preventive surgery before developing breast cancer, removing the breast tissue to reduce the risk of cancer development.*

Risk of Malignancy in Individuals with a Germline *BRCA1* or *BRCA2*-Pathogenic Variant.

Cancer Type	General Population Risk	Risk for Malignancy [1]	
		BRCA1	***BRCA2***
Breast	12%	46%-87%	38%-84%
Second primary breast	2% w/in 5 yrs	21.1% w/in 10 yrs; 83% by age 70	10.8% w/in 10 yrs; 62% by age 70
Ovarian	1%-2%	39%-63%	16.5%-27%
Male breast	0.1%	1.2% Risk increased ten folds	Up to 8.9%
Prostate	6% through age 69	8.6% by age 65	15% by age 65; 20% lifetime
Pancreatic	0.50%	1%-3%	2%-7%
Melanoma (cutaneous & ocular)	1.6%		Elevated risk

https://www.ncbi.nlm.nih.gov/books/NBK1247/

Table 1.

Molecular Genetic Testing Used in *BRCA1* and *BRCA2* Associated Hereditary Breast/Ovarian Cancer (HBOC)

Gene [1]	Proportion of *BRCA1/BRCA2*-Associated HBOC Attributed to Pathogenic Variants in Gene	Proportion of Pathogenic Variants [2] Detected by Method	
		Sequence analysis [3]	Gene-targeted deletion/duplication analysis [4]
BRCA1	66%	>80% [5]	~10% [5]
BRCA2	34%	>80% [5]	~10% [5]

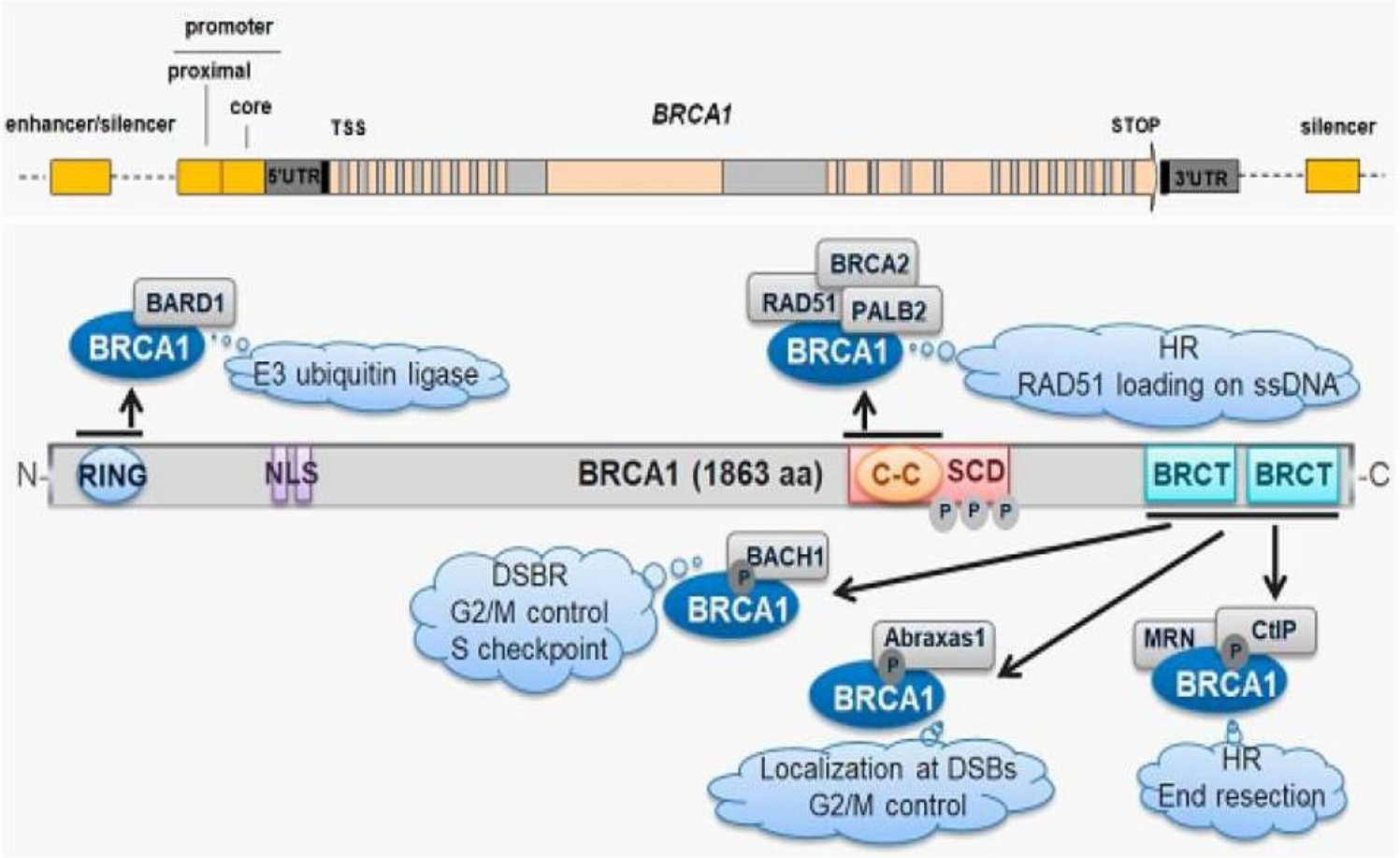
- **More than 80%** of BRCA1/BRCA2 mutations are single-nucleotide variants, nonsense mutations, and small insertions or deletions (indels). For example, a 5-nucleotide deletion within a BRCA exon can cause a frameshift mutation, resulting in a truncated protein that loses its function. **Around 10%** of mutations are large deletions or duplications, typically involving sequences of 1 kb (1000 bases) or more; these may include deletion of the entire gene or duplication of part of it, and are known as ***copy number variants*** *(CNVs)*. Since BRCA1/BRCA2 are tumor suppressor genes, loss-of-function mutations impair their role in controlling cell division and maintaining genomic stability, thereby increasing the risk of cancer development.

- Moreover, mutations in BRCA2 and, to a lesser extent, BRCA1 , also account for 10% to 20% of all male breast cancer and increase the risk for male breast cancer ten to sixtyfold over the 0.1% lifetime risk observed among males in the general population

- The gene products of BRCA1 and BRCA2 are nuclear proteins contained within the same multiprotein complex. This complex has been implicated in the cellular **response to double-stranded DNA breaks** and correct them (repair), such as occur:

✓ normally during homologous recombination between the non sister chromatids of homologous chromosomes, so BRCA 1 and BRCA 2 reform phosphodiester bonds & covalent bonds (note: the bonds between DNA strands are hydrogen bonds)

✓ abnormally as a result of damage to DNA.



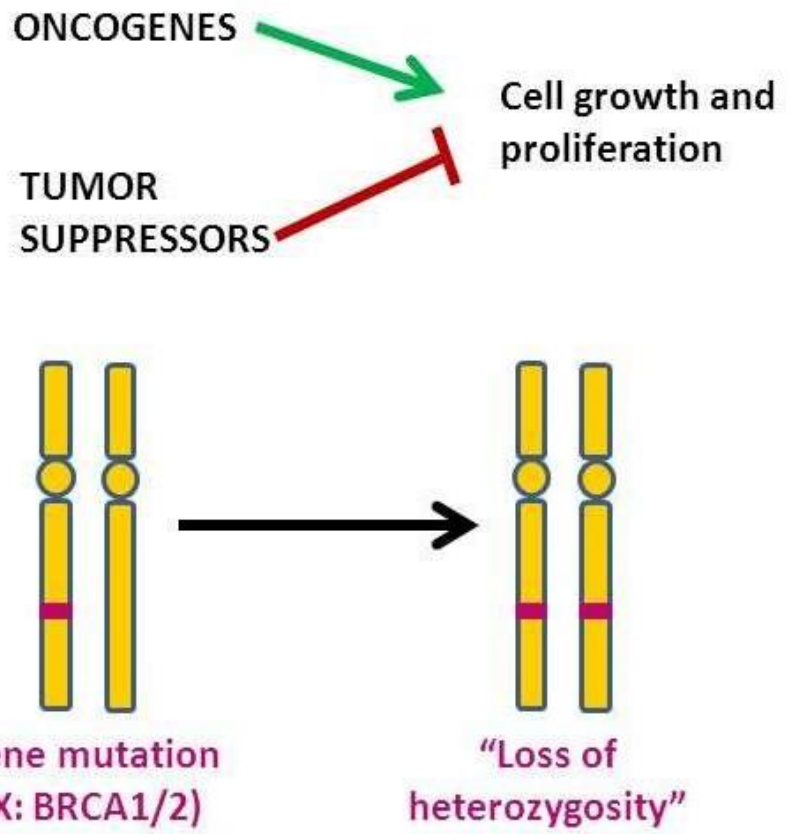
Sporadic vs. Familial Cancer

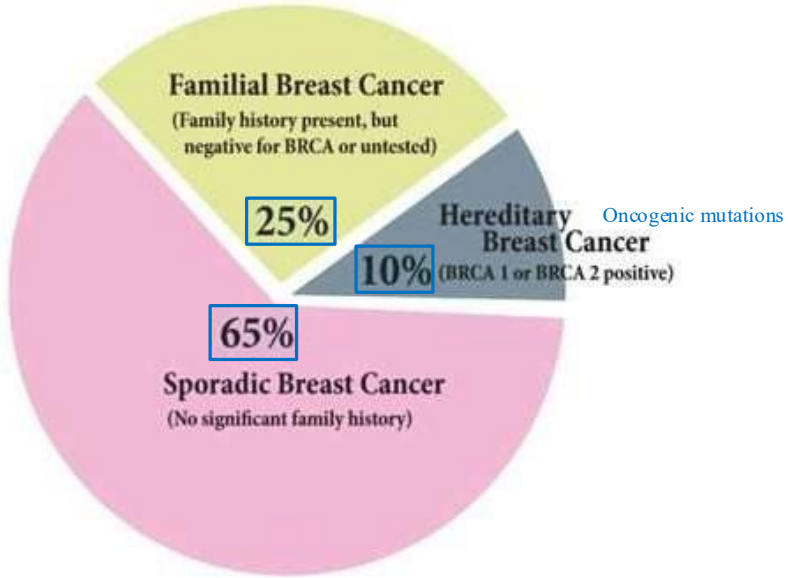
- As might be expected for any TSG, tumor tissue from **heterozygotes** for BRCA1 and BRCA2 mutations frequently demonstrates LOH with loss of the normal allele.

Sporadic = Mutation-driven

Familial = Inherited mutation + "loss of heterozygosity"

Somatic mutation





Oncogenic mutations

The second normal copy may undergo a **somatic mutation**. Somatic mutation can be a stop codon, missense mutation, splice site mutation, but it can also be ***loss of heterozygosity***, which is a common mechanism for the second hit.

Penetrance of BRCA1 and BRCA2 Mutations

- Presymptomatic detection of women at risk for development of breast cancer as a result of any of these susceptibility genes relies on detecting clearly pathogenic mutations by **gene sequencing.**

✓ Susceptible genes imply that if an individual (female) has a mutation in **BRCA1** or **BRCA2**, she is susceptible and has a higher risk of developing breast cancer. However, this does not mean a 100% risk of developing the disease.

- For the purposes of patient management and counseling, it would be helpful to know the lifetime risk for development of breast cancer in individuals, whether male or female, carrying particular mutations in the BRCA1 and BRCA2 genes, compared with the risk in the general male or female population

Breast Cancer Panels

Primary panel (14 genes)

There are genes other than BRCA1 and BRCA2 that are involved in breast cancer.

ATM	BARD1	BRCA1	BRCA2	BRIP1	CDH1	CHEK2	NBN
NF1	PALB2	PTEN	RAD50	STK11	TP53		

- *This is a group of genes with established gene–disease validity, meaning these genes are known to cause or increase the risk of breast cancer.*

ABRAXAS1	AKT1	FANCC	FANCM	MRE11	MUTYH	PIK3CA	RAD51C
RAD51D	RECQL	RINT1	SDHB	SDHD	XRCC2		

Breast Cancer - Comprehensive Risk Panel

Summary and Pricing

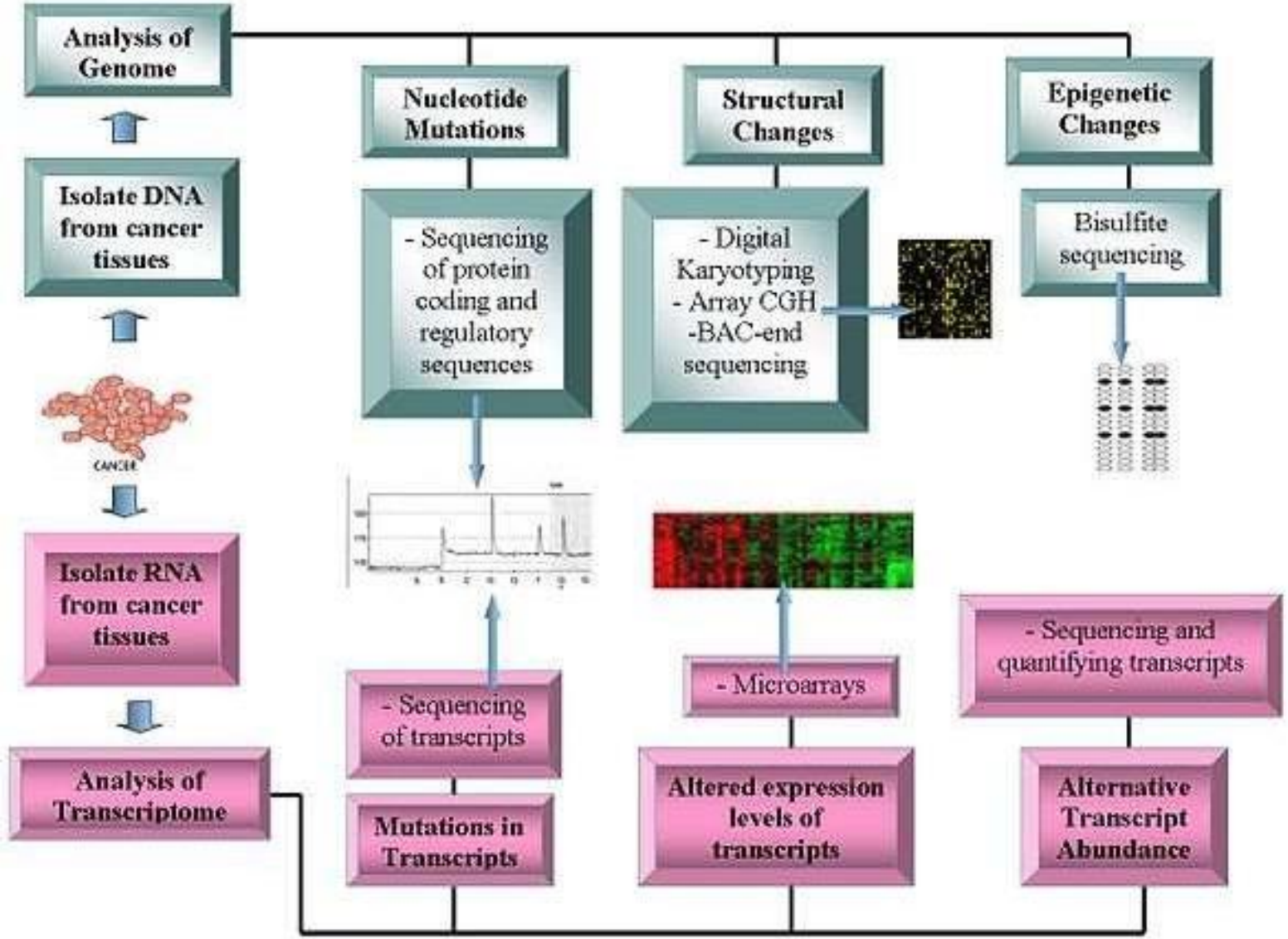
Test Method

Sequencing and CNV Detection via NextGen Sequencing using PG-Select Capture Probes

New York State Approved Test

Test Code	Test (Copy Genes)	Panel CPT Code	Gene CPT Codes (Copy CPT Code)	Base Price	
5435	Genes x (18)	81479	81162, 81307, 81321, 81323, 81404, 81405, 81406, 81408, 81479	$540	Order Options and Pricing

- There are genetic testing companies in America, such as **INVITAE, GeneDx, and Blueprint Genetics**, as well as clinically validated laboratories and hospitals that provide established genetic testing services.
- For example, in a female with a family history of breast cancer, a good starting point is either testing BRCA1/BRCA2 or using a multigene panel. This is because patients with familial breast cancer often have recurrent pathogenic mutations in this group of genes.


Analysis of Genome
Isolate DNA from cancer tissues
CANCER
Isolate RNA from cancer tissues
Analysis of Transcriptome
Nucleotide Mutations
- Sequencing of protein coding and regulatory sequences
- Sequencing of transcripts
Mutations in Transcripts
Structural Changes
- Digital Karyotyping
- Array CGH
-BAC-end sequencing
- Microarrays
Altered expression levels of transcripts
Epigenetic Changes
Bisulfite sequencing
- Sequencing and quantifying transcripts
Alternative Transcript Abundance

Hereditary Colon Cancer

- Colon cancer can be **hereditary (germline) or somatic.**

- Colorectal cancer, a malignancy of the **epithelial** cells of the colon and rectum, is one of the *most common* forms of cancer.

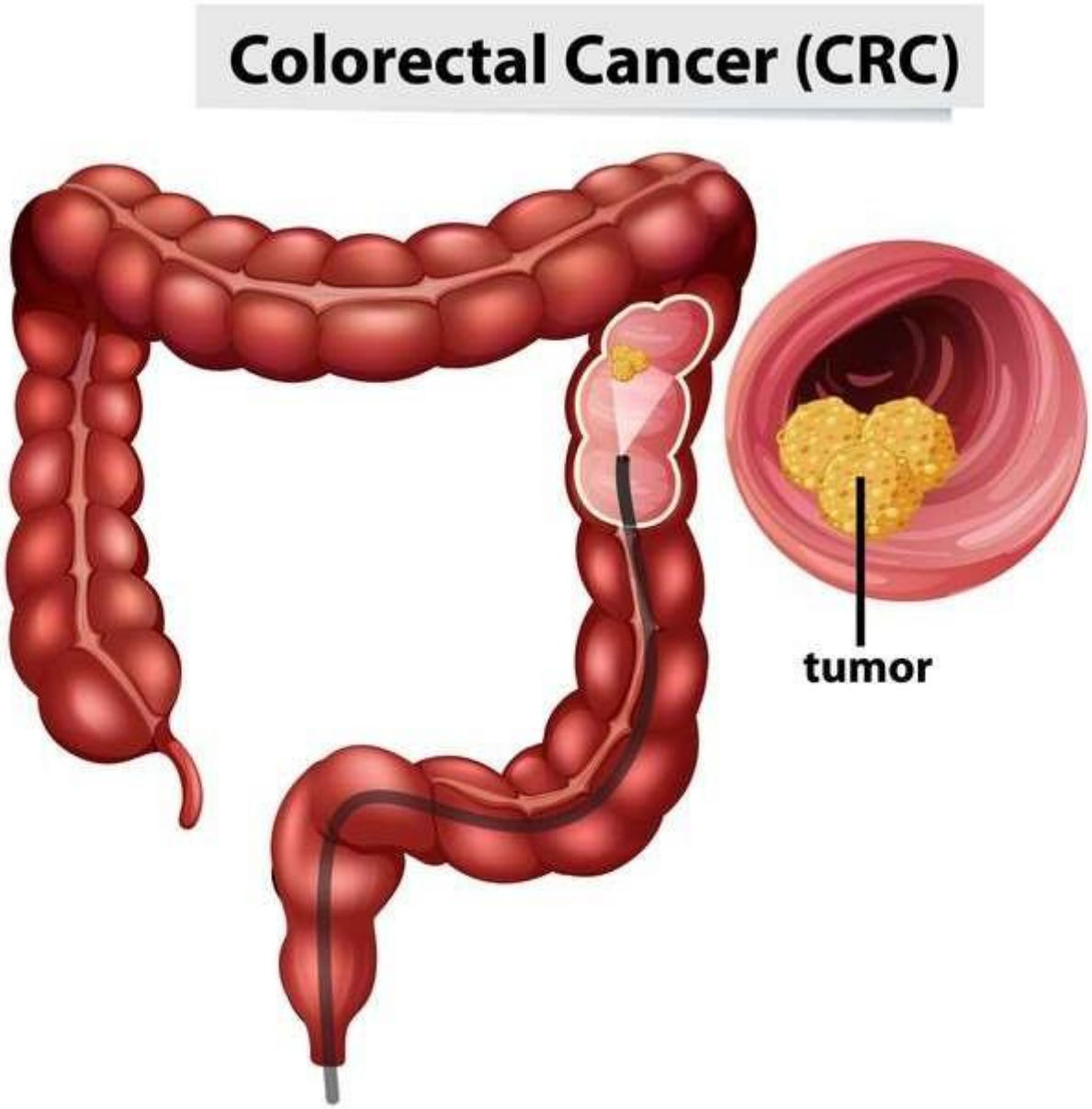
- It is responsible for approximately 10% to 15% of all cancer.

- Most cases are sporadic (No family history), however a small proportion of colon cancer cases are familial, among which are **two autosomal dominant conditions**:

❑ familial adenomatous polyposis (FAP)

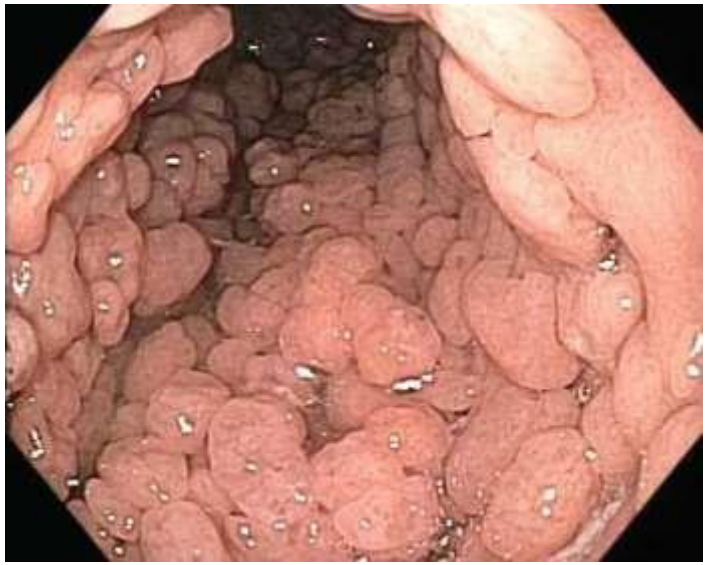
❑ Lynch syndrome (LS)

along with their variants.

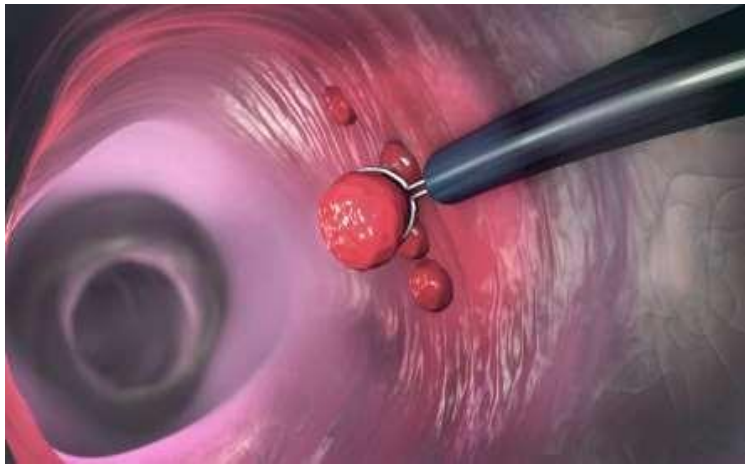


Familial Adenomatous Polyposis (FAP)

- FAP and its subvariant, **Gardner syndrome**, together have an incidence of approximately 1 per 10,000. (It's a high figure)
- In FAP heterozygotes, benign adenomatous polyps numbering in the many hundreds develop abnormally in the colon during the first two decades of life.
- In almost all cases, one or more of the polyps becomes malignant.
- Surgical removal of the colon (colectomy) prevents the development of malignancy.



- If an individual is tested for **Familial Adenomatous Polyposis (FAP)** —either due to a family history or the presence of multiple benign polyps —and a **pathogenic mutation** is identified, then this result has important clinical implications.
- Regarding genetic testing, if a properly classified pathogenic mutation is confirmed, clinical decisions such as colectomy **(removal of the colon)** may be considered based on the genetic result



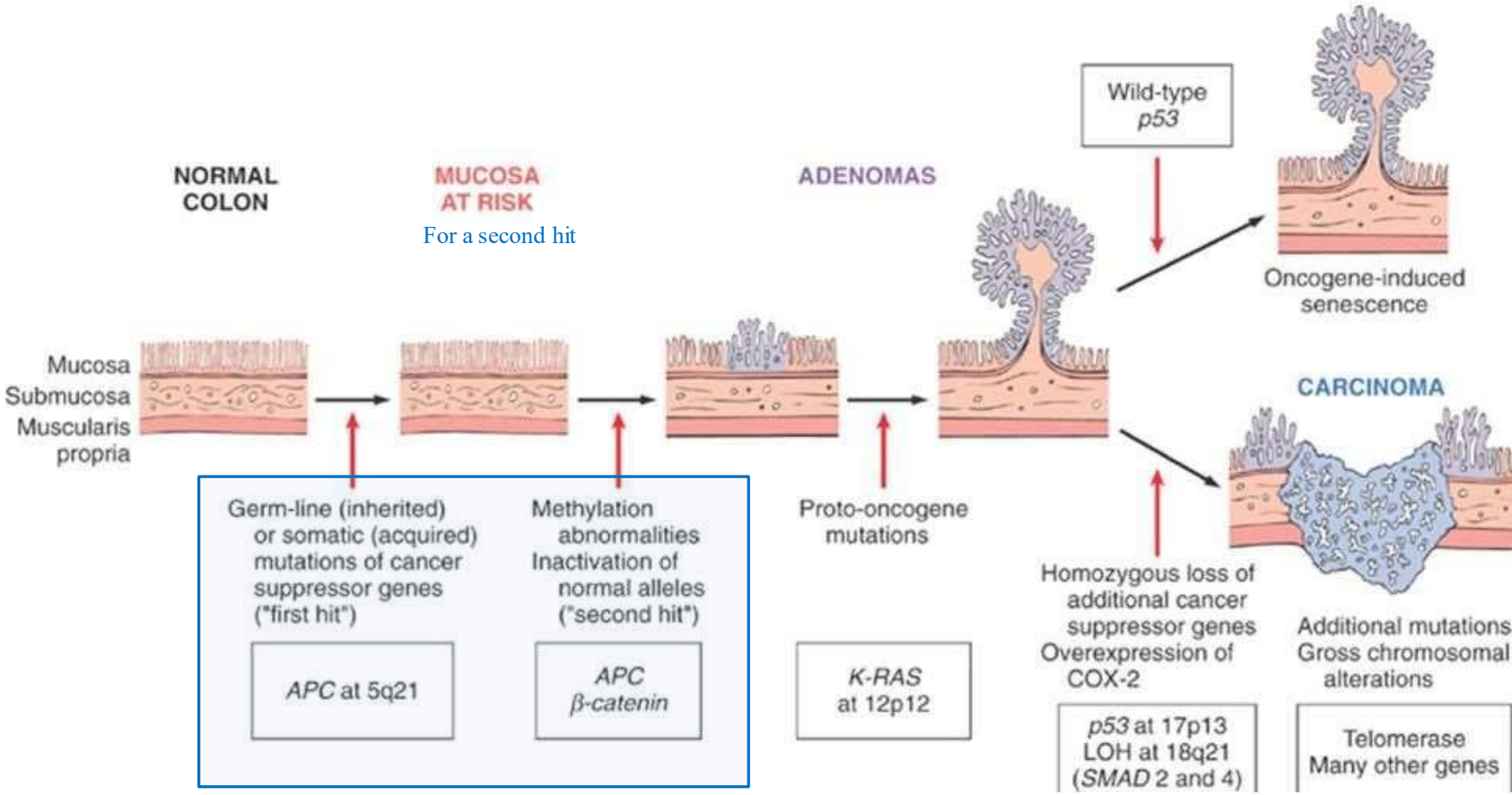
FAP is caused by loss-of-function mutations in a TSG known as the **APC** gene

(so-named because the condition used to be called **adenomatous polyposis coli**).

Multi-step Carcinogenesis

Single Oncogene cannot transform cells

Multiple genetic alterations involving both the activation of many oncogenes and loss of more then one tumor suppressor genes are necessary for carcinogenesis



- A mutation in a cell can cause it to start growing and dividing faster than normal, leading to the accumulation of additional mutations over time. As these genetic alterations build up, the cell acquires pathogenic changes involving oncogenes such as **KRAS**, resulting in the formation of an adenoma. With further accumulation of mutations, particularly in tumor suppressor genes (TSGs), the lesion can progress from adenoma to carcinoma. This stepwise accumulation of mutations is seen in conditions such as **Familial Adenomatous Polyposis (FAP)**.

Gardner syndrome is a variant of FAP

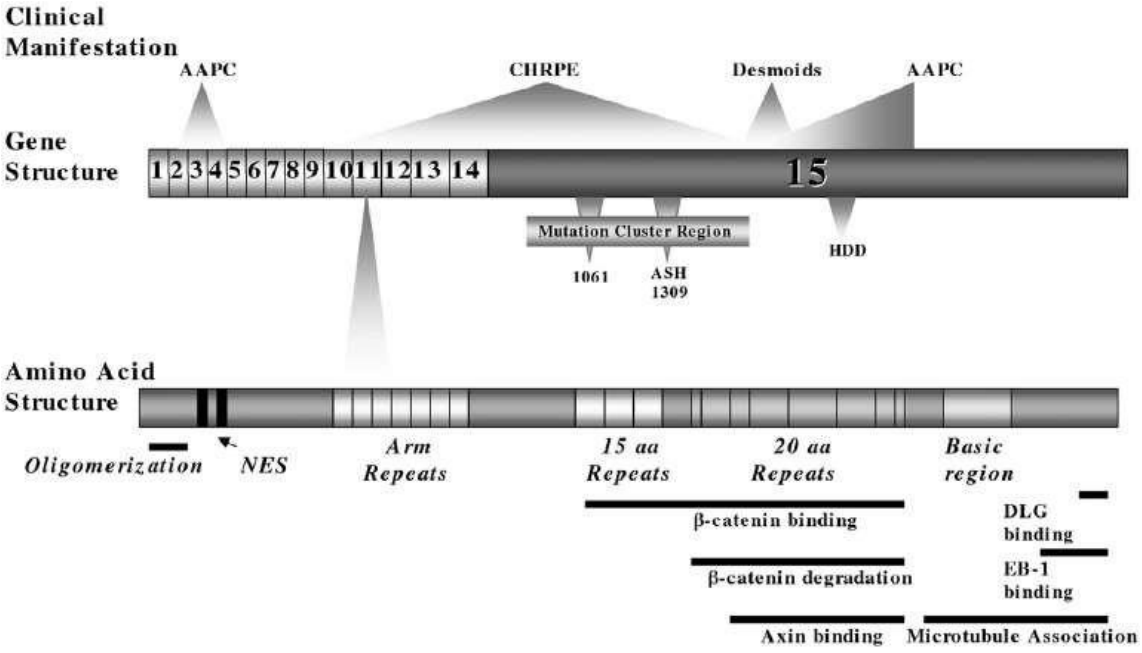
- Gardner syndrome is also due to mutations in APC and is therefore allelic to FAP.
- Gardner syndrome is a form of familial FAP that is characterized by multiple colorectal polyps and various types of tumors, both benign and malignant.
- People affected by Gardner syndrome have a high risk of developing colorectal cancer at an early age (earlier onset)
- **Extra colonic tumors:** Patients with Gardner syndrome have, in addition to the adenomatous polyps with malignant transformation seen in FAP, other extracolonic anomalies, including osteomas of the jaw and desmoids, which are tumors arising in the muscle of the abdominal wall.

Gardner syndrome is a variant of FAP

- Although the relatives of an individual affected with Gardner syndrome who also carry **the same APC mutation** tend to also show the extracolonic manifestations of Gardner syndrome, the same mutation in unrelated individuals has been found to cause only FAP in one individual and Gardner syndrome in another.

- Thus whether or not an individual has FAP or Gardner syndrome is not simply due to which mutation is present in the APC gene but is likely affected by genetic variation elsewhere in the genome.

  - It may also be due to **epistatic effects**, where mutations in other genes can influence disease development, leading in some cases to only FAP, while in others resulting in earlier onset and additional manifestations.



Characteristics of different forms of FAP

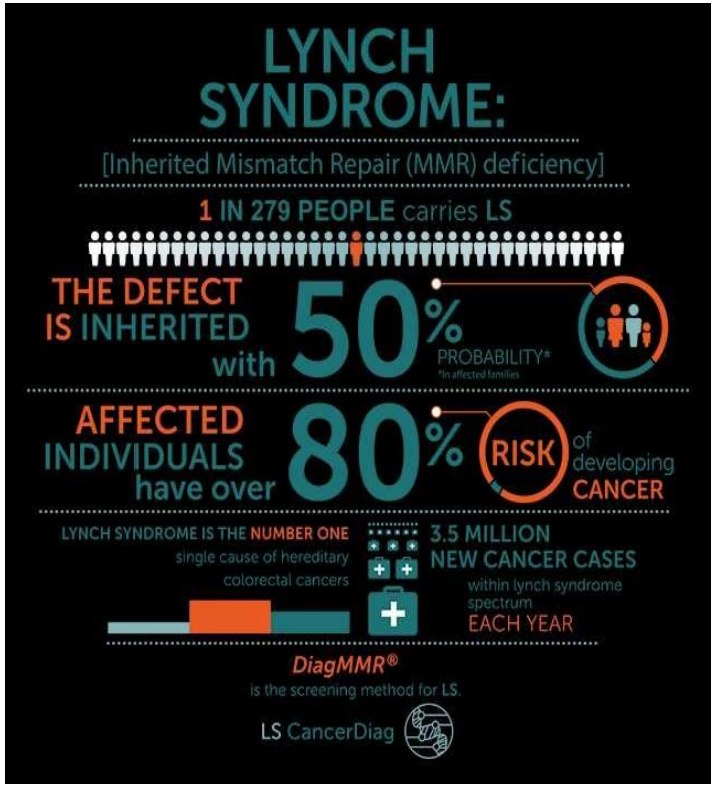
	Classical form of FAP	Attenuated form of FAP**	Gardner syndrome	Turcot syndrome
Gene	APC; 5q21	APC; 5q21 N-terminal mutation	APC; 5q21	APC; 5q21
Transmission *	Autosomal dominant	Autosomal dominant	Autosomal dominant	Autosomal dominant
Colic manifestations	Adenomatous polyps	Adenomatous polyps, late onset, low number	Adenomatous polyps	Adenomatous polyps
Extra-colic manifestations	Absent	Absent	HCRPE, epidermoid cysts, pilomatricoma, fibrous hyperplasia, desmoid tumour, multiple osteomas, various digestive tumours and extra-digestive manifestations	Brain tumour: glioblastoma, medulloblastoma, basal cell carcinoma, brown spots

*The transmission is autosomal dominant in inheritance, but recessive at the cellular level due to the two-hit hypothesis (recall lecture 5)

**Attenuated FAP is essentially a milder (less severe) variant of classic FAP.

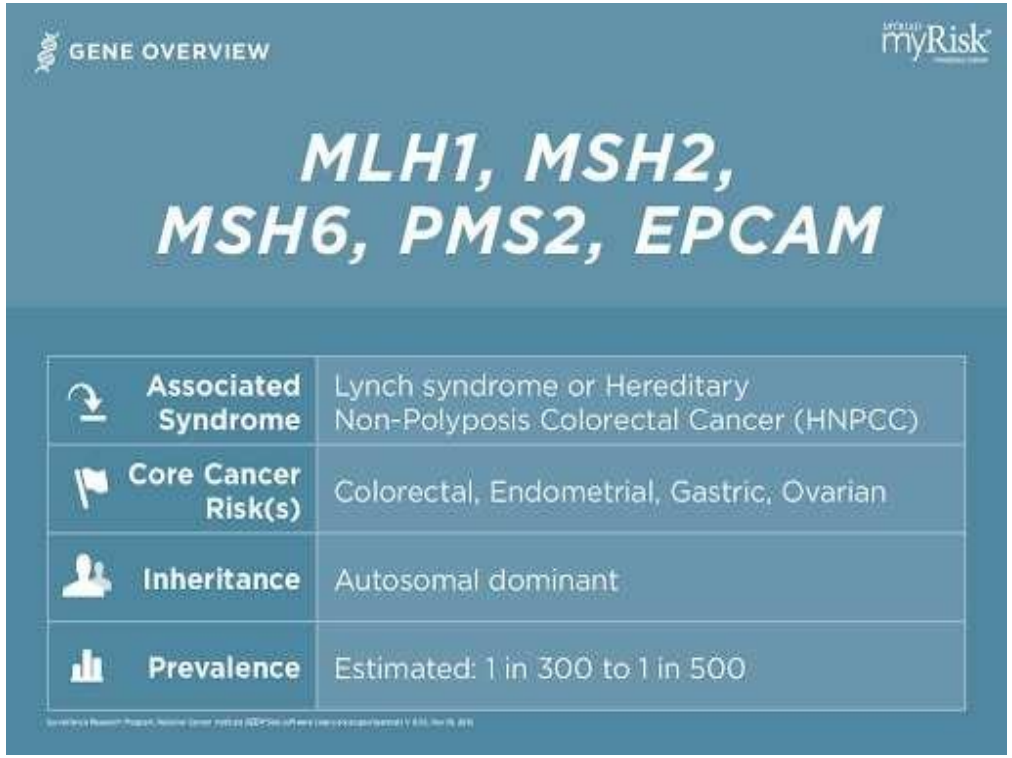
Lynch Syndrome (LS)

- 2% to 4% of cases of colon cancer are attributable to LS
- LS is characterized by autosomal dominant inheritance of colon cancer in association with a small number of adenomatous polyps that begin during early adulthood
- The number of polyps is generally quite small, in contrast to the hundreds to thousands of adenomatous polyps seen with FAP
- the polyps in LS have high potential to undergo malignant transformation.
- Heterozygotes for the most commonly mutated LS gene have an approximately 80% lifetime risk for development of cancer of the colon;
- female heterozygotes have a somewhat smaller risk (approximately 70%) but also have an approximately 40% risk for endometrial cancer.



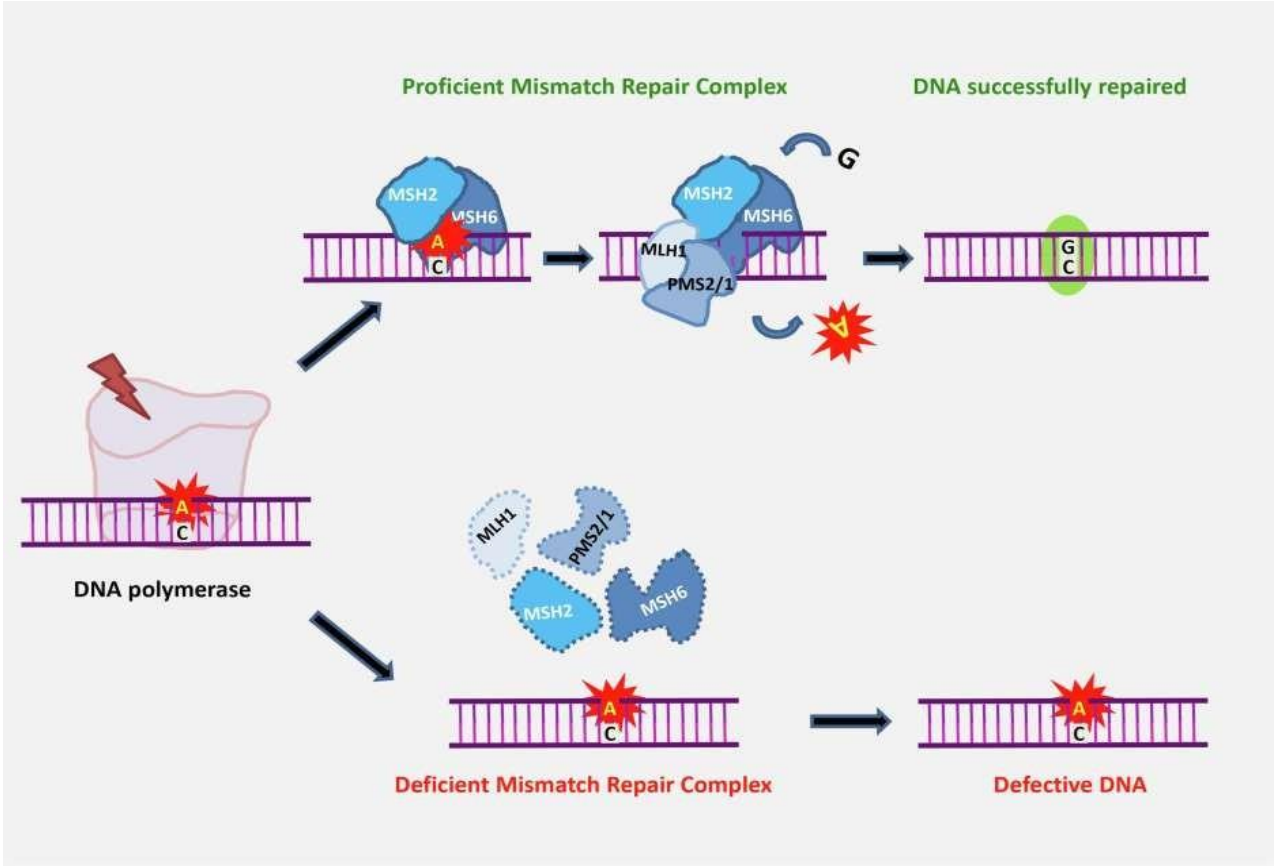
Lynch Syndrome (LS)

- LS results from loss-of-function mutations in one of four distinct but related DNA repair genes (MLH1, MSH2, MSH6, and PMS2) that encode mismatch repair proteins so the mismatch repair system becomes deficient, leading to accumulation of replication errors and **microsatellite instability**, which is a hallmark of Lynch syndrome.
- Although all four of these genes have been implicated in LS in different families, MLH1 and MSH2 are together responsible for the vast majority of LS, whereas the others have been found in only a few patients and are often associated with a lesser degree of mismatch repair deficiency and lower penetrance.
- **Lynch syndrome** can be suspected clinically from **colorectal** or **endometrial** cancer, OR confirmed by **genetic testing** showing mutations in mismatch repair genes. If mutated, diagnosis is followed by **preventive management**, including possible **surgical intervention**, to reduce cancer risk.



Lynch Syndrome (LS)

- Like the BRCA1 and BRCA2 genes, the LS mismatch repair genes are TSGs involved in maintaining the integrity of the genome.
- Unlike BRCA1 and BRCA2 , however, the LS genes are not involved in double-stranded DNA break repair. Instead, their role is to repair incorrect DNA base pairing (i.e., pairing other than A with T or C with G) that can arise during DNA replication.

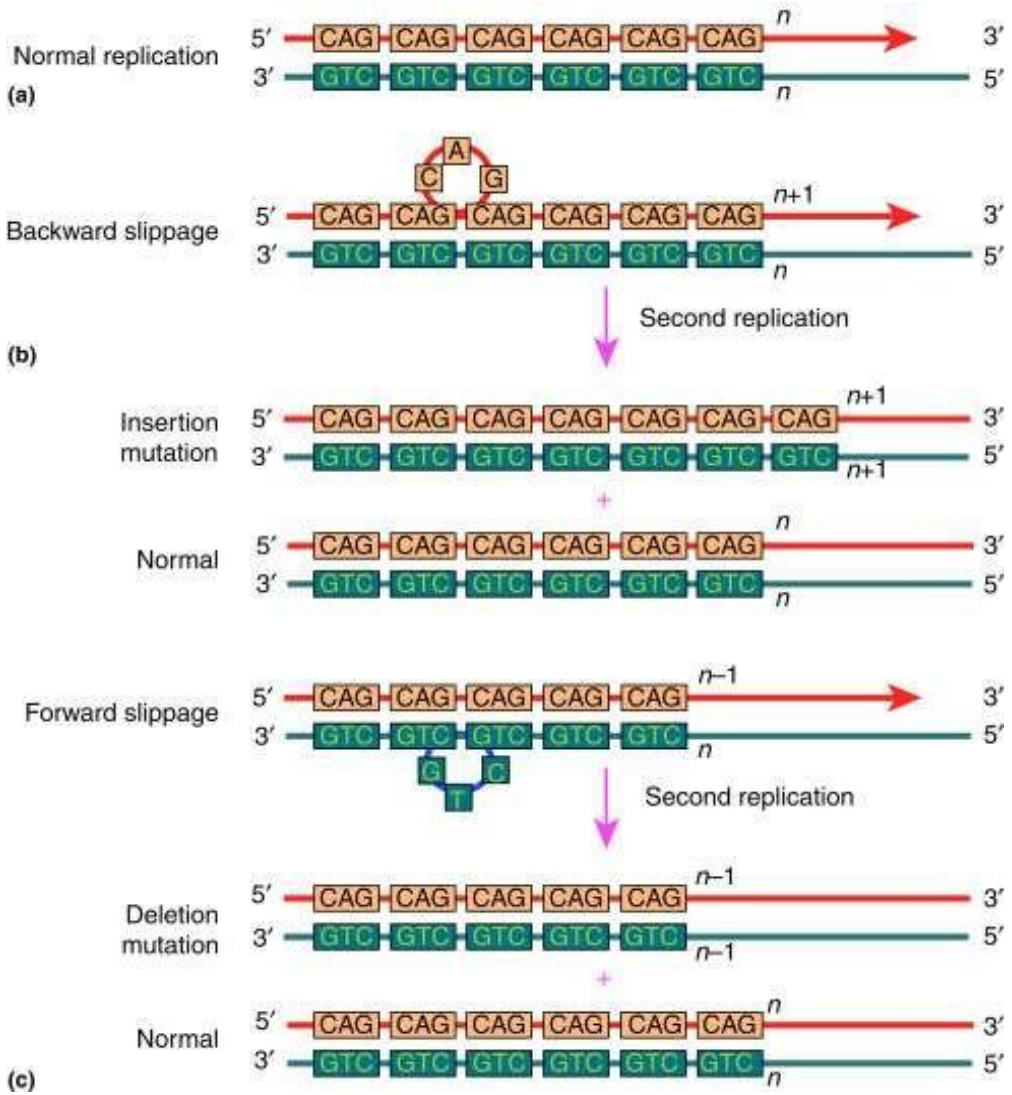


Lynch Syndrome (LS)

At the cellular level, the most striking phenotype of cells lacking mismatch repair proteins is an enormous increase in both:

- point mutations and
- mutations occurring during replication of simple DNA repeats, such as a segment containing a string of the same base, for example (A) n , or a microsatellite, such as (TG) n.

Microsatellites are believed to be particularly vulnerable to mismatch because slippage of the strand being synthesized on the template strand can occur more readily when a short tandem repeat is being synthesized



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Lynch Syndrome (LS)

- If DNA replication errors are not repaired, mutations accumulate progressively over time. With continued accumulation of mutations —especially in **tumor suppressor genes** and **oncogenes**— cells may undergo transformation from normal tissue into malignant tissue.

- Certain DNA regions are naturally harder for DNA polymerase to replicate accurately, particularly **repetitive sequences**. These include: **Homopolymer repeats** → AAAAAAAA (same nucleotide repeated many times) OR **Microsatellites** → short tandem repeats such as **CAG-CAG-CAG**

- During replication of these repetitive regions, DNA polymerase can slip (**replication slippage**). This may cause: **Backward slippage** → insertion → extra repeat (**n+1**) OR
- **Forward slippage** → deletion → missing repeat (**n−1**)

- As a result, the newly synthesized strand may contain a different number of repeats than the template strand. Normally, **DNA mismatch repair (MMR) proteins** correct these insertion/deletion errors after replication. This is especially important in repetitive DNA regions where the error rate is higher.

- In Lynch syndrome, mutations in MMR genes impair this repair mechanism. Because these errors are no longer corrected, mutations accumulate—particularly within microsatellite regions—leading to **microsatellite instability (MSI)**, which is a hallmark of Lynch syndrome.

Lynch Syndrome (LS)

- This instability, is referred to as the **microsatellite instability-positive (MSI+)** phenotype occurs at two orders of magnitude higher frequency in cells lacking both copies of a mismatch repair gene.
- The MSI+ phenotype is easily seen in DNA as three, four, or even more alleles of a microsatellite polymorphism in a single individual's tumor DNA.
- It is estimated that cells lacking both copies of a mismatch repair gene may carry 100,000 mutations within simple repeats throughout the genome

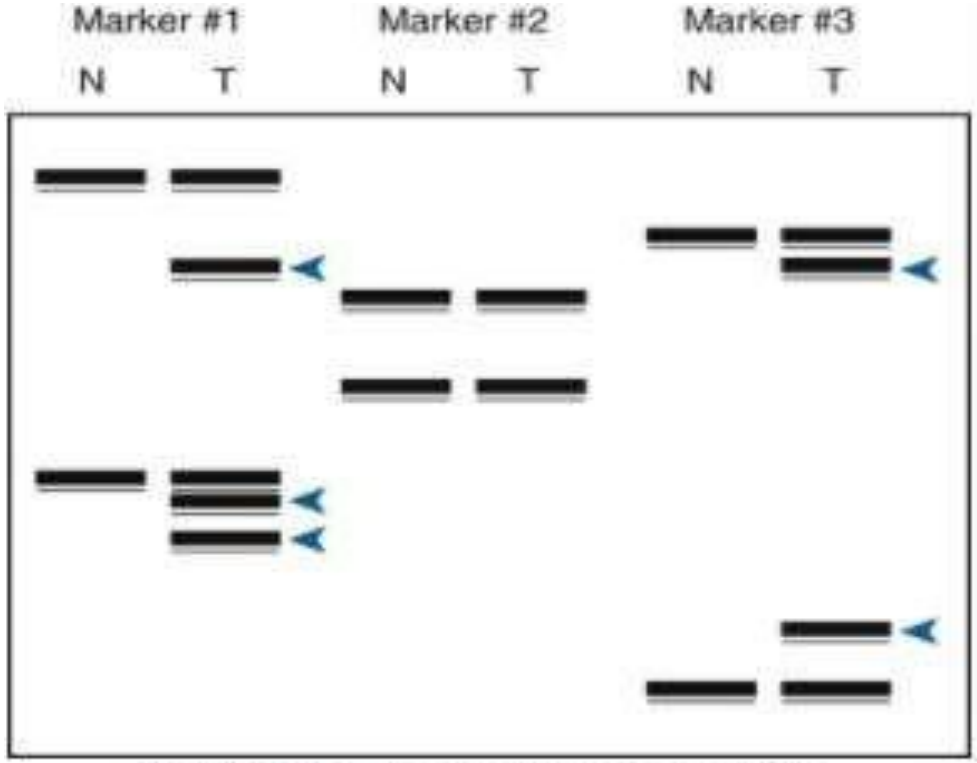


FIGURE 15-9 Gel electrophoresis of three different microsatellite polymorphic markers in normal (N) and tumor (T) samples from a patient with a mutation in *MSH2* and microsatellite instability. Although marker #2 shows no difference between normal and tumor tissues, genotyping at markers #1 and #3 reveals extra alleles (*blue arrows*), some smaller, some larger, than the alleles present in normal tissue.

Examine the figure carefully then see the next slide

Lynch Syndrome (LS)

- In this figure, **N** represents **normal tissue** and **T** represents **tumor tissue**. Each band corresponds to a **microsatellite marker** analyzed in that DNA sample. In this example, three different markers are tested.

- These markers are selected from specific regions of the genome that contain **repetitive DNA sequences**, such as **CAG repeats** or dinucleotide repeats like **TG-TG-TG**.

- Under normal conditions, the number of repeats in these regions is stable, so normal tissue shows bands of a predictable size. However, when **mismatch repair (MMR) genes** are mutated —as in Lynch syndrome— errors that occur during DNA replication are not corrected. DNA polymerase may slip while copying these repetitive sequences, leading to: **Insertion** of extra repeats (**expansion**) OR **Deletion** of repeats (**shrinkage**)

- This means the newly synthesized DNA strand may contain a different number of repeats compared with the original template strand. When these regions are amplified by **PCR** and compared between normal tissue and tumor tissue, the PCR products differ in length because the number of repeats has changed.

- Therefore, the **tumor band** may appear at a different position than the **normal band**, because the fragment size is different. AND even within the tumor, different groups of cells may accumulate different repeat lengths, creating multiple bands.
- This change in microsatellite length between normal and tumor DNA is called **microsatellite instability (MSI)**.

Lynch Syndrome (LS)

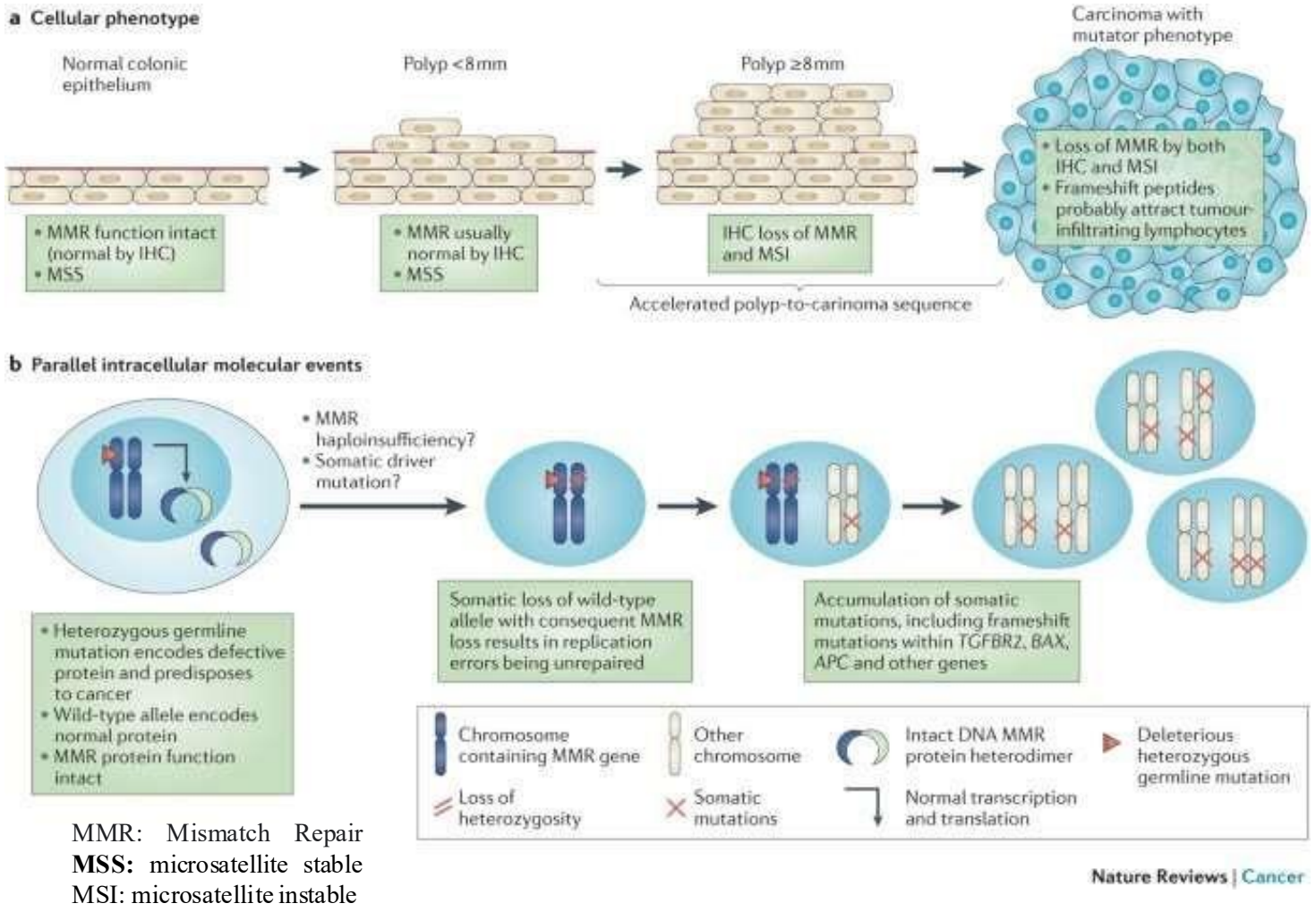
- Because one of the **mismatch repair (MMR) genes** is defective, these tumors are estimated to carry **thousands of mutations**. This indicates widespread genomic instability. The mutations are not limited to repetitive microsatellite regions; they may also affect **coding regions of other important genes**, including **tumor suppressor genes** and **oncogenes**, which further promote tumor development.

- Because of the increase in mutation rate in these classes of sequence, loss of function of mismatch repair genes will lead to somatic mutations in other driver genes.

- Two such driver genes have been isolated and characterized:

- The first is APC, whose normal function and role in FAP were described previously.

- The second is the gene TGFBR2 , in which mutations also cause an autosomal dominant hereditary colon cancer syndrome.



MMR: Mismatch Repair
MSS: microsatellite stable
MSI: microsatellite instable

Lynch Syndrome (LS)

A) Progression from normal tissue to carcinoma

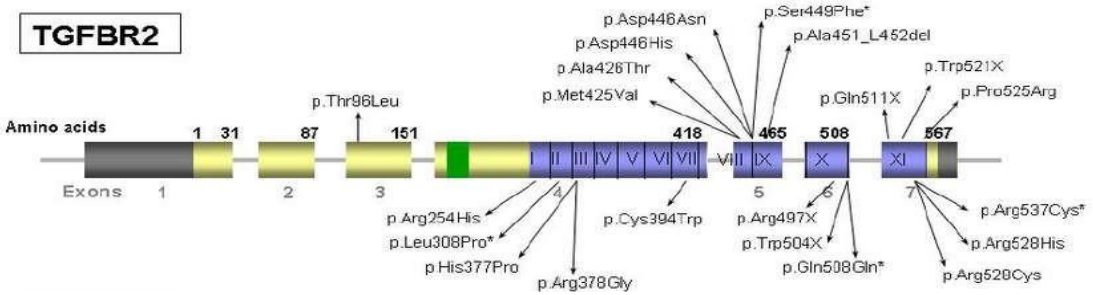
- Panel **A** represents normal colonic epithelial tissue in which the mismatch repair system is functioning normally. Therefore, **microsatellites remain stable (MSS: microsatellite stable)**. If the microsatellite markers are compared between normal tissue and tumor tissue at this stage, they appear similar in size.
- Later, a **polyp (adenoma)** begins to develop. In this polyp, mismatch repair becomes defective, causing **microsatellite instability (MSI)**. Because replication errors are no longer repaired, mutations progressively accumulate.
- With further accumulation of mutations, the lesion progresses to **carcinoma with a hypermutated phenotype**. At this stage there is **loss of mismatch repair protein expression on immunohistochemistry (IHC)** and **microsatellite instability is detectable**, leading ultimately to malignant transformation.

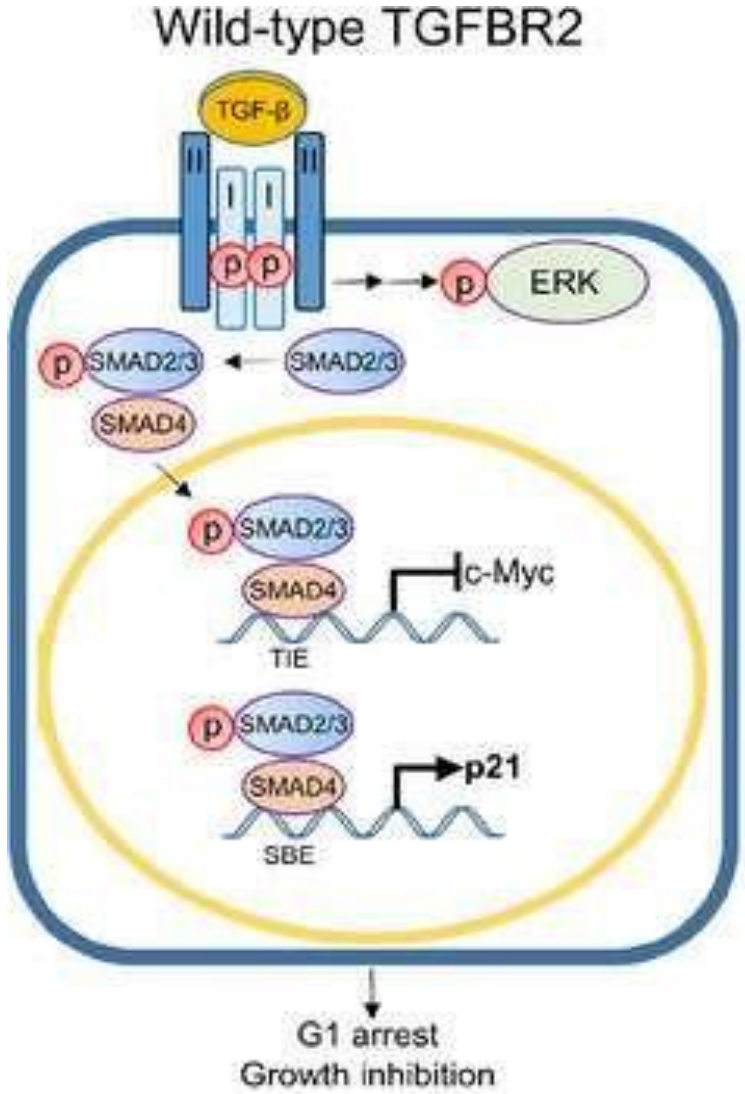
B) Two-hit mechanism in Lynch syndrome

- Panel **B** shows how a **heterozygous germline mutation** in one mismatch repair gene predisposes an individual to cancer. Initially, one allele is mutated in the germline and the second allele remains normal. Because one normal allele is still present, mismatch repair function remains intact.
- Then a **second hit** occurs, causing **loss or inactivation of the normal allele**. Once both alleles are lost/inactivated, mismatch repair function is deficient. As a result, replication errors are no longer repaired and mutations accumulate throughout the genome
- These somatic mutations can affect important **driver genes**, such as: **TGFβ receptor genes**, **BAX**, **TP53** and other oncogenes/tumor suppressor genes. Accumulation of these mutations drives progression from normal mucosa to adenoma and eventually to **malignant tumor transformation**.

Lynch Syndrome (LS)

- TGFBR2 encodes transforming growth factor β receptor II, a serine-threonine kinase that inhibits intestinal cell division.
- TGFBR2 , is particularly vulnerable to mutation when mismatch repair proteins are lost because it contains a stretch of 10 adenines encoding three lysines within its coding sequence
- deletion of one or more of these As results in a frameshift and loss-of-function mutation.
- LS is an excellent example of how a gene, like MLH1 , which has a global effect on mutation rate throughout the genome, can be a driver gene through its effect on other genes, such as TGFBR2 , that are more specifically involved in driving the development of a cancer.





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Lynch Syndrome (LS)

The **TGFB pathway** involves the TGF-β receptor, a membrane-bound kinase receptor that regulates cell growth and inhibits intestinal epithelial cell proliferation. When **TGF-β ligand binds to its receptor**, it activates receptor-mediated phosphorylation signaling. This triggers downstream signaling involving **SMAD2/3 proteins**, which become phosphorylated and then move into the nucleus. There, they regulate gene expression by: **Suppressing MYC**, a proto-oncogene that promotes cell cycle progression and proliferation AND **Inducing p21**, a tumor suppressor protein that inhibits the cell cycle.
Overall, this pathway acts as a **growth-inhibitory mechanism** in intestinal cells.

However, mutations in the **TGF-β receptor (e.g., TGFBR2)**—can disrupt this regulation. These mutations are more likely to accumulate when **DNA mismatch repair (MMR) is defective**, as seen in microsatellite instability (MSI). When the receptor is mutated, it may lose its kinase function and fail to transmit the signal. As a result:

- MYC is no longer suppressed
- p21 is not induced
- Cell cycle inhibition is lost
- Cells continue to proliferate and accumulate further mutations

The **exon structure of the TGF-β receptor gene (TGFBR2)** shows specific regions where mutations commonly occur, one important example is a mutation in **exon 3**, where a **missense mutation** occurs, such as **p.Thr96Leu**. When this missense mutation occurs, **loss of function of the TGF-β signaling pathway is seen**.

In addition to missense mutations, other types of pathogenic alterations in this gene include **Truncating mutations**, **Nonsense mutations**, which introduce a premature stop codon or **Splice-site mutations.**