cancer is fundamentally a genetic disease II

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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, bilateral disease or second independent primary breast tumor, and second primary cancers in other tissues such as ovary and prostate.

Breast cancer → hereditary : germline, runs in the family.
→ non-hereditary : somatic, does NOT run in the family & occurs due to spontaneous mutations in the breast tissue

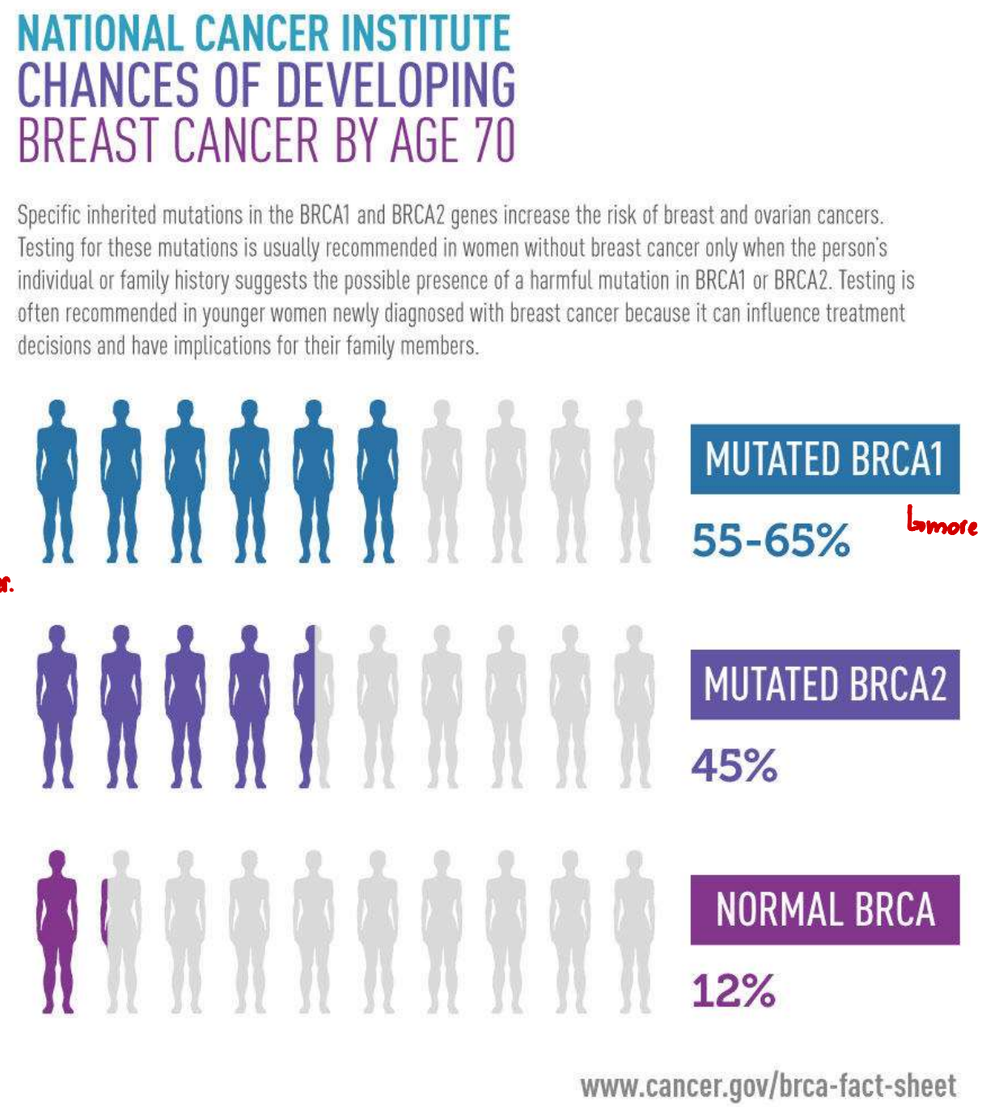
TSGs
BRCA1 and BRCA2 are responsible for the majority of all hereditary breast cancers

Together, these two TSGs account for approximately one **half** (BRCA1) and one **third** (BRCA2), respectively, of autosomal dominant familial breast cancer. ⇒ BRCA1 is more common than BRCA2.

(NCBI: Breast **cancer risks** are at
40–87% for **BRCA1 mutation carriers**
18–88% for **BRCA2 mutation carriers**.
} they have comparable risks with BRCA1 slightly higher.

For ovarian **cancer**, the **risk** estimates are in the range of:
22–65% for **BRCA1**
10–35% for **BRCA2**.
} BRCA1 has a notably higher risk of causing ovarian cancer than 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%.

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%	Up to 8.9% → Risk of male breast cancer is higher for BRCA2.
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/

* that's why many women with BRCA1 & BRCA2 pathogenic variants decide to undergo propylactic mastectomy (before developing breast cancer to avoid its risk)

TSGs

* If a female carries a germline mutation (disease-causing variant), with time the chance for a 2nd hit increases. A 2nd hit is required to develop disease manifestations.

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]

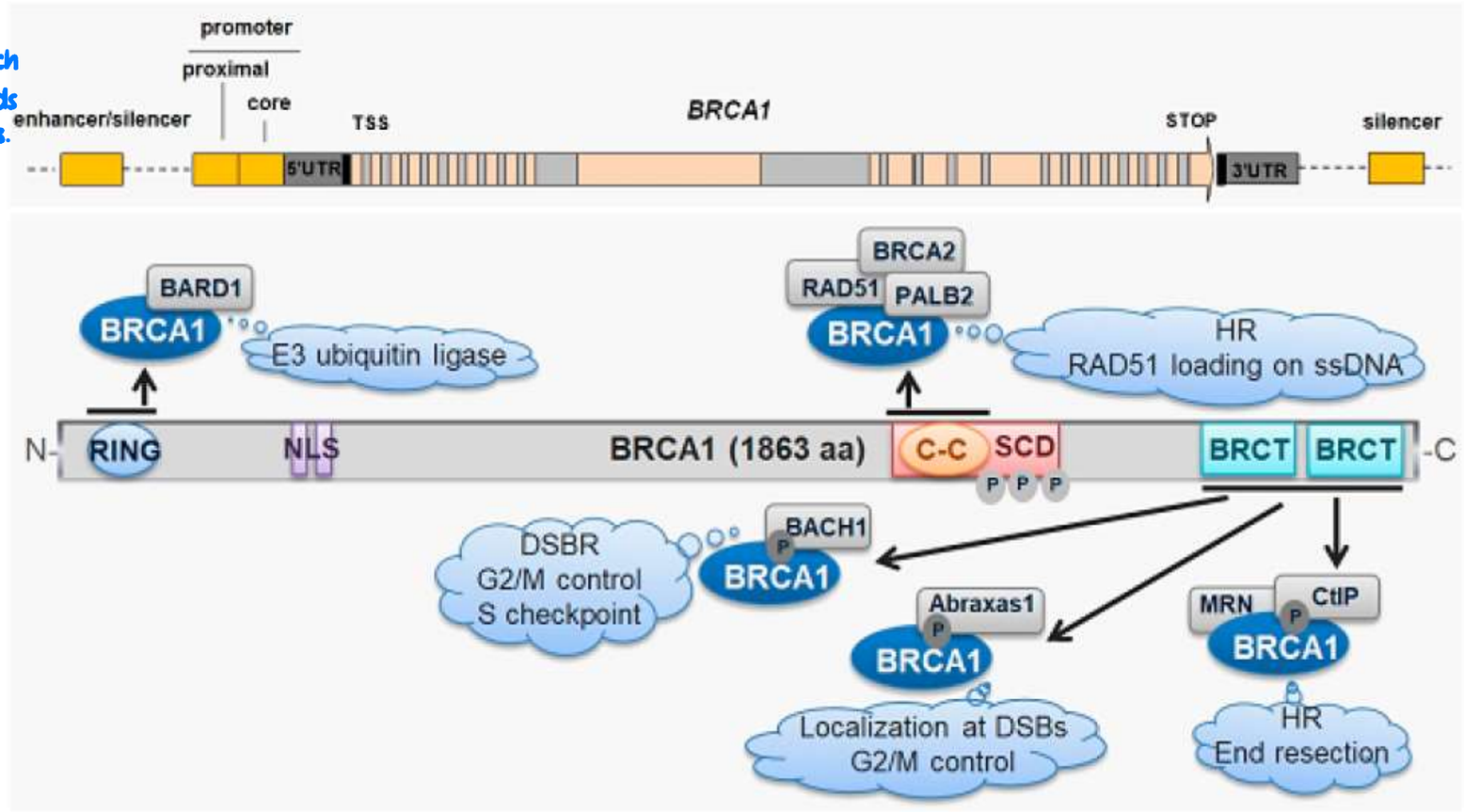
Moreover, mutations in BRCA2 and, to a lesser extent, BRCA1 , also account for 10% to 20% of all male breast cancer and increase the risk for male breast cancer ten to sixtyfold over the 0.1% lifetime risk observed among males in the general population

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**, such as occur normally during homologous recombination or abnormally as a result of damage to DNA.

↳ BRCA1 & BRCA2 play a role in dsDNA break repair.

*between non-sister chromatids of homologous chromosomes

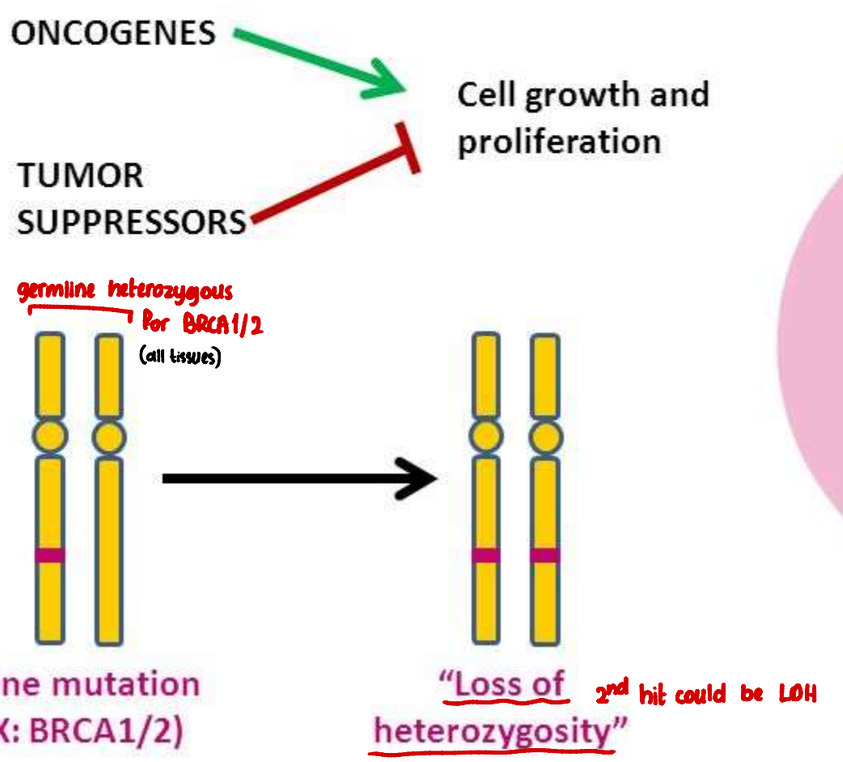
*normally causes dsDNA breaks which will then re-form phosphodiester bonds & covalent bonds between the strands. (by BRCA1/2)

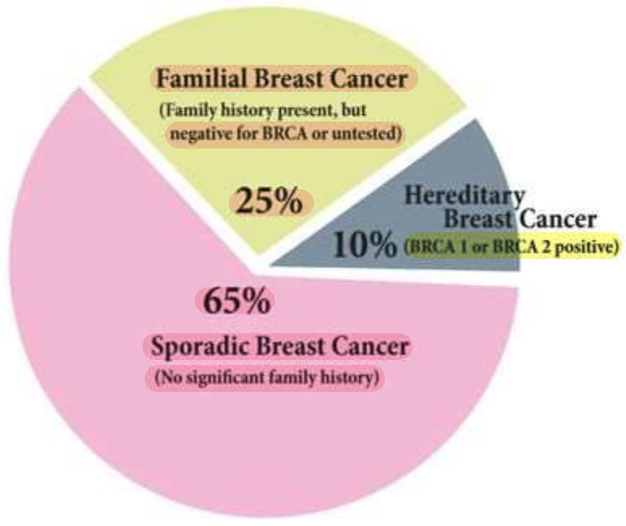


As might be expected for any TSG, tumor tissue from heterozygotes for BRCA1 and BRCA2 mutations frequently demonstrates LOH with loss of the normal allele.

Sporadic vs. Familial Cancer

Sporadic = Mutation-driven
Familial = Inherited mutation + "loss of heterozygosity"





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or The individual is heterozygous in the germline (in all tissues) → the 2nd normal copy/allele undergoes a somatic mutation
(could be of any type, i.e: stop codon, missense, splice site mutation BUT it could also be LOH which is a common mechanism for the 2nd 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.

* this means that if a female has BRCA1/2 variant (susceptible, at higher risk of developing breast cancer BUT not 100% risk) then it would be helpful to acknowlege the lifetime risk & compare it with the general population for the same type of cancer.

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

* there are genes other than BRCA1/2 that are involved in breast cancer.

INVITAE ↱ genetic testing company
(other companies: GeneDx, Blueprint Genetics)

Breast Cancer Panels → they are a group of genes that have established gene-disease validity (they are known to increase breast cancer risk)

Primary panel (14 genes)

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

NF1: also causes Neurofibromatosis.
PTEN: causes Cowden disease & breast cancer.
TP53: TSG

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

Breast Cancer - Comprehensive Risk Panel

* Some hospitals provide genetic testing services.

Summary and Pricing

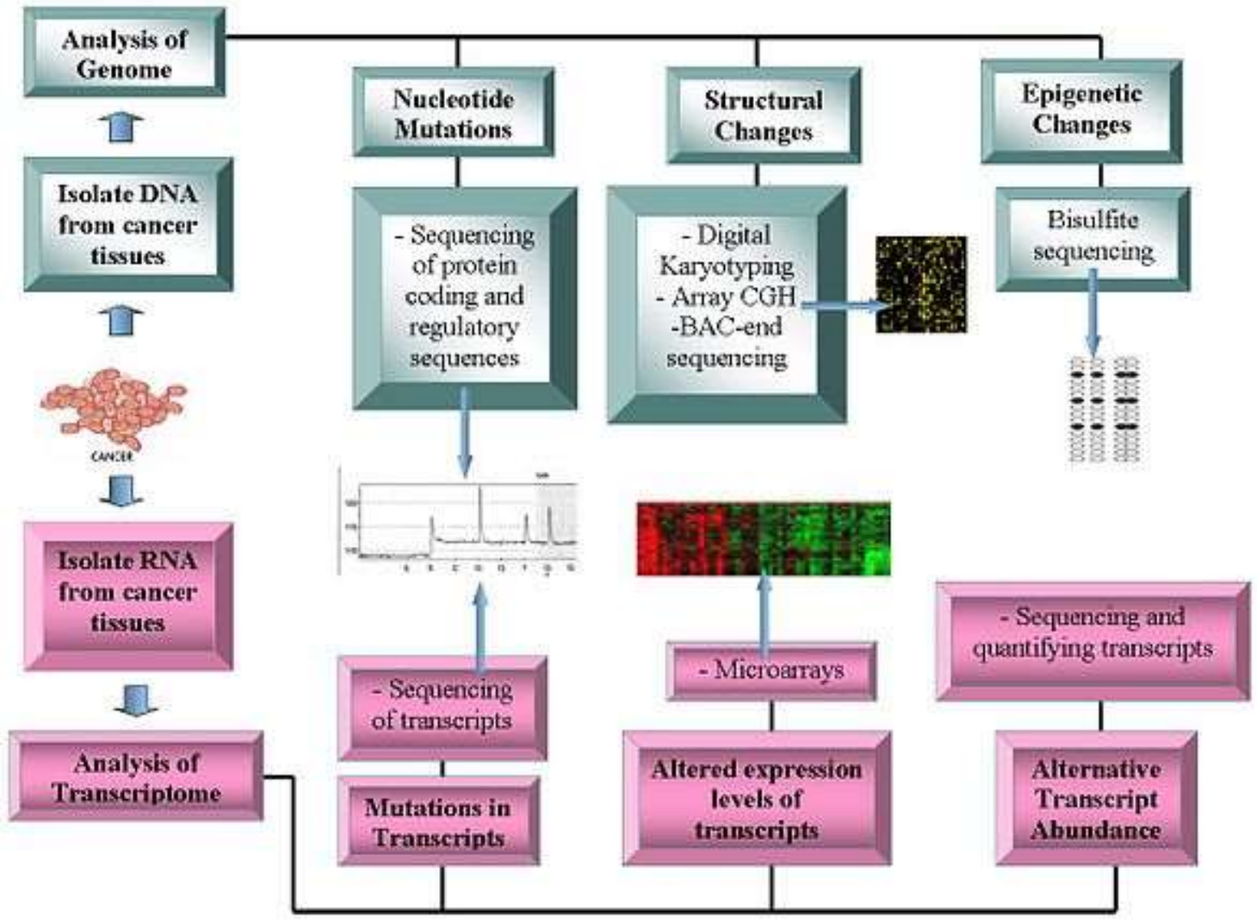
Test Method

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

i.e: if a female came with a family history of breast cancer, then genetic testing for BRCA1/2 (or even the panel above) would be a good starting point.


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

* Colon cancer could be → somatic
                        → hereditary

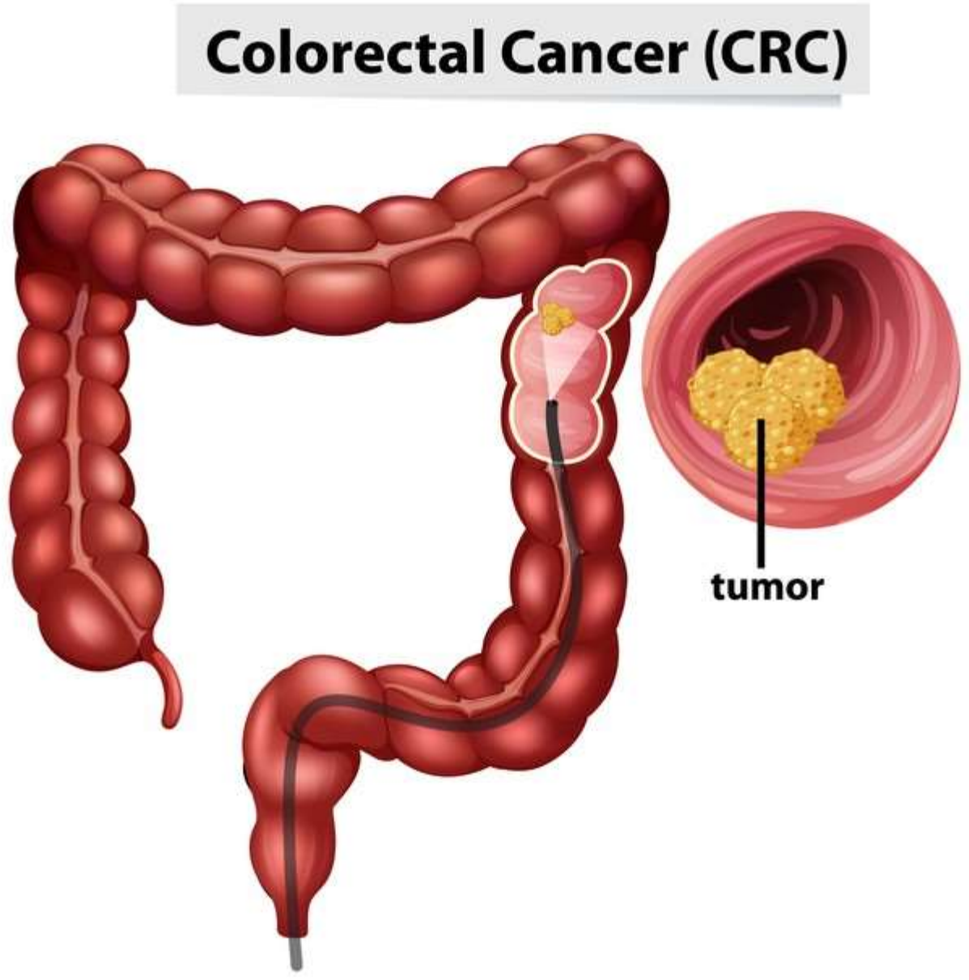
Hereditary Colon Cancer

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.

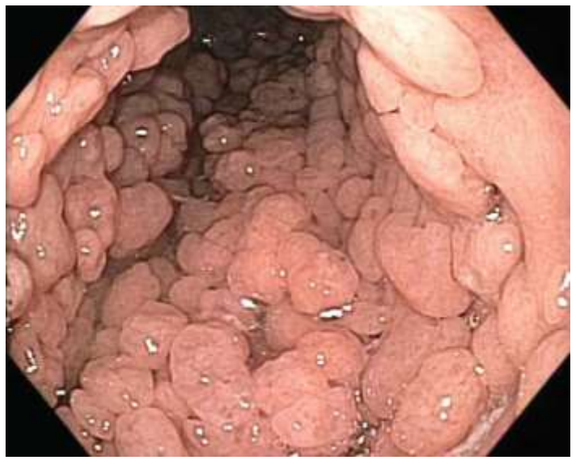
Most cases are sporadic

a small proportion of colon cancer cases are familial, among which are two autosomal dominant conditions: familial adenomatous polyposis (FAP) and 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.

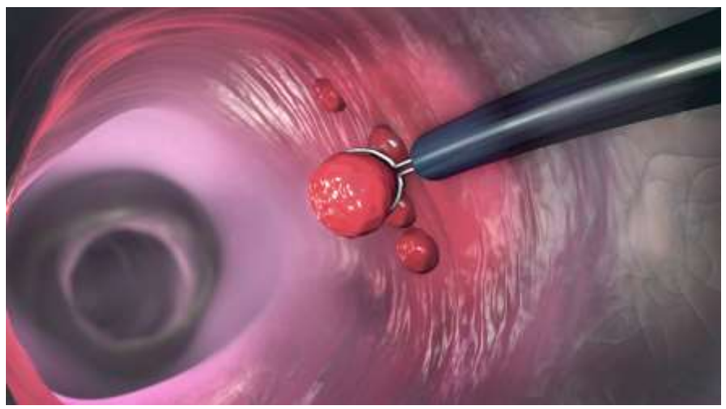


In FAP heterozygotes, benign adenomatous polyps numbering in the many hundreds develop in the colon during the first two decades of life.

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 came to test for APC (either bcz of family hx, or appearance of benign polyps), proper variant classification is very critical bcz a decision like colectomy can be made based on genetic testing.



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

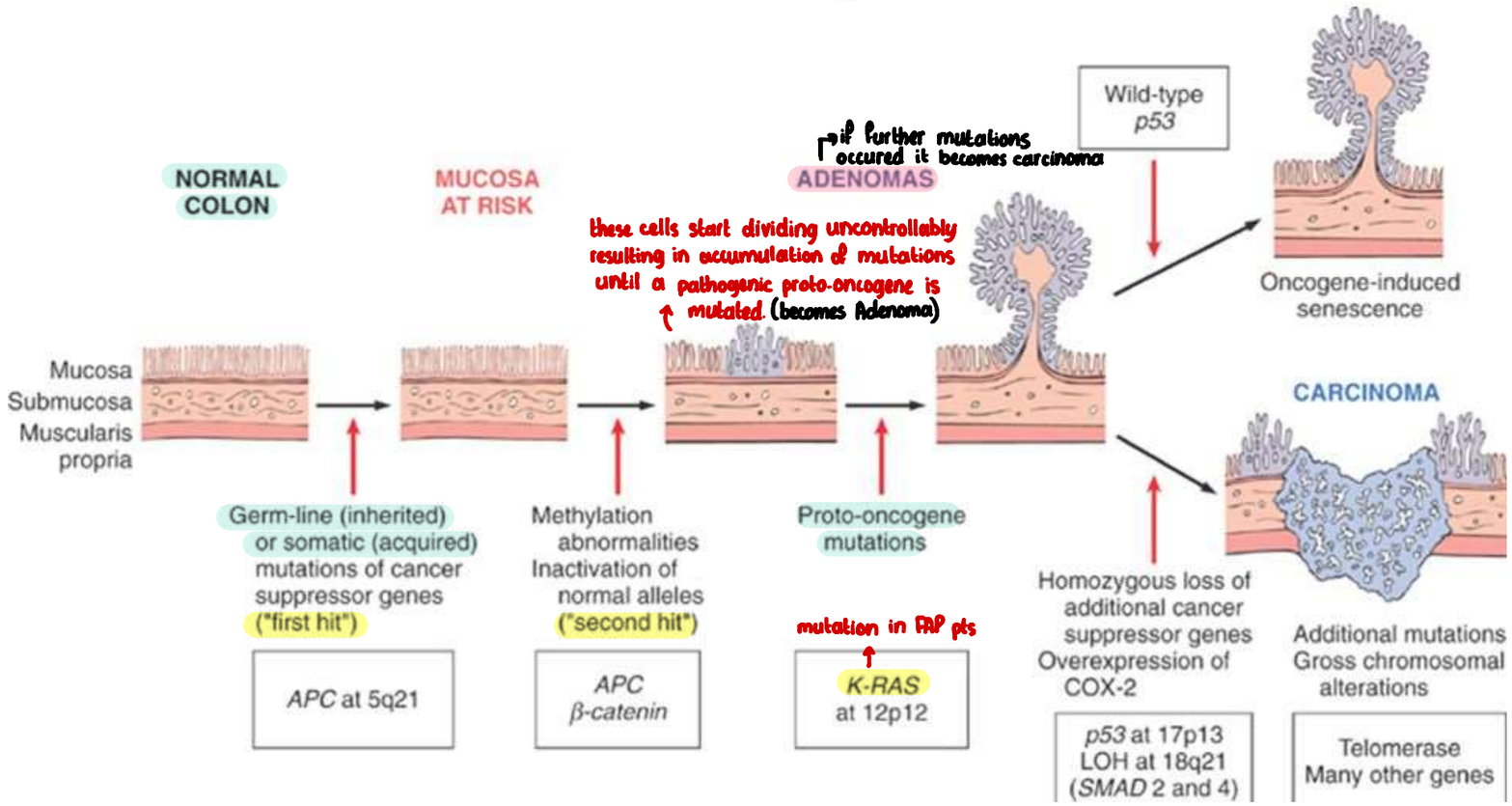
Adenomatous Polyposis Coli

(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



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

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.

Q: Why do some individuals develop Gardner Syndrome which is more severe with earlier onset while others develop FAP only, even though the same gene is involved in both?

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,

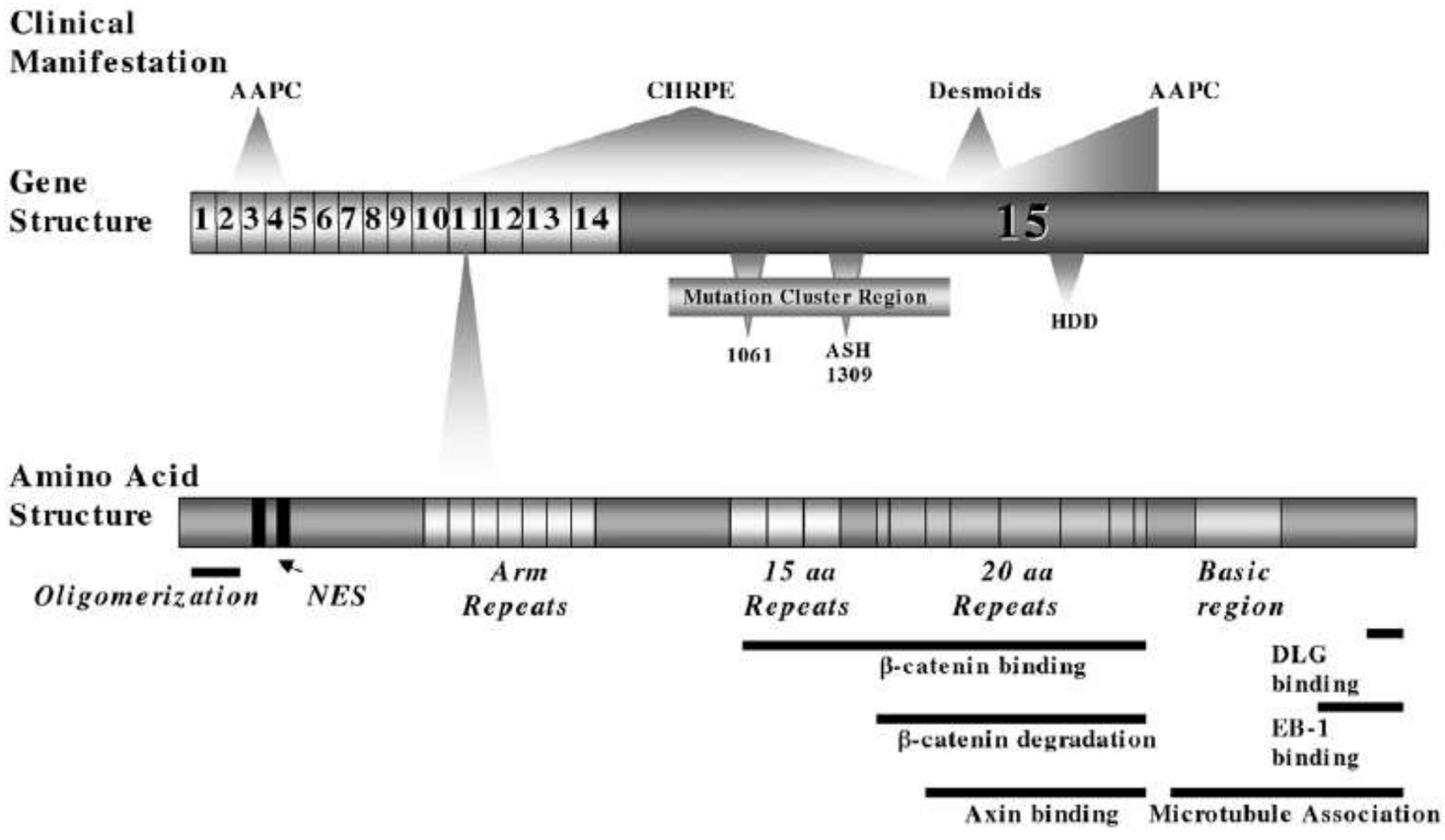
the same mutation in unrelated individuals has been found to cause only FAP in one individual and Gardner syndrome in another.

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.

↓

"epistatic effect"

i.e: other mutation somewhere else in the genome impacts FAP development only of Gardner Syndrome.



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 ①	Autosomal dominant	Autosomal dominant	Autosomal dominant
Colic manifestations	Adenomatous polyps	Adenomatous polyps, late onset, low number ②	Adenomatous polyps	Adenomatous polyps
Extra-colic manifestations	Absent	Absent	✓extracolic manifestations HCEPR, epidermoid cysts, pilomatrixoma, fibrous hyperplasia, desmoid tumour, multiple osteomas various digestive tumours and extra digestive	Brain tumour: glioblastoma, medulloblastoma. Basal cell carcinoma. Brown spots.

① on the genetic level → recessive (1st hit then 2nd hit)

② a mutation affecting the N-terminus results in less severe manifestations & late onset.

Lynch Syndrome (LS)

2% to 4% of cases of colon cancer are attributable to LS

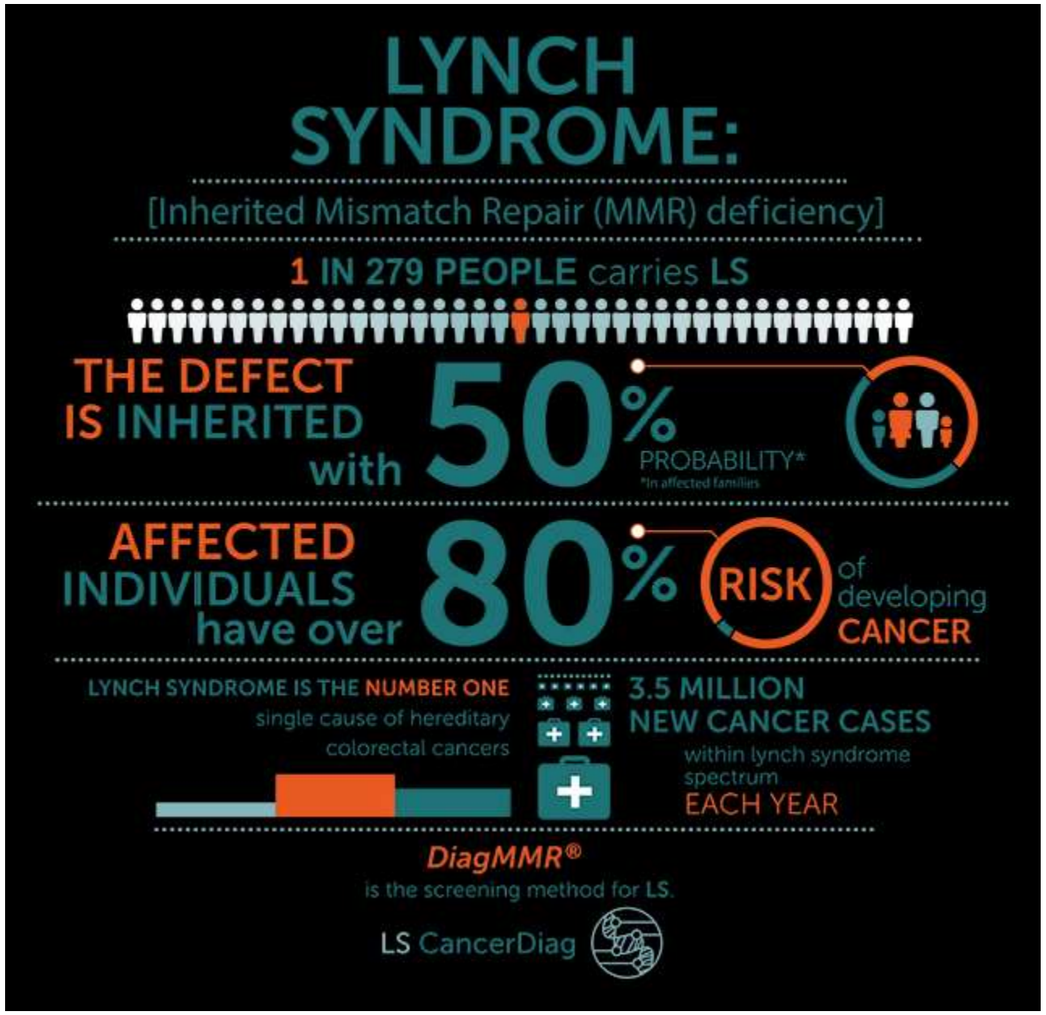
LS is characterized by autosomal dominant inheritance of colon cancer in association with a small number of adenomatous polyps that begin during early adulthood

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.

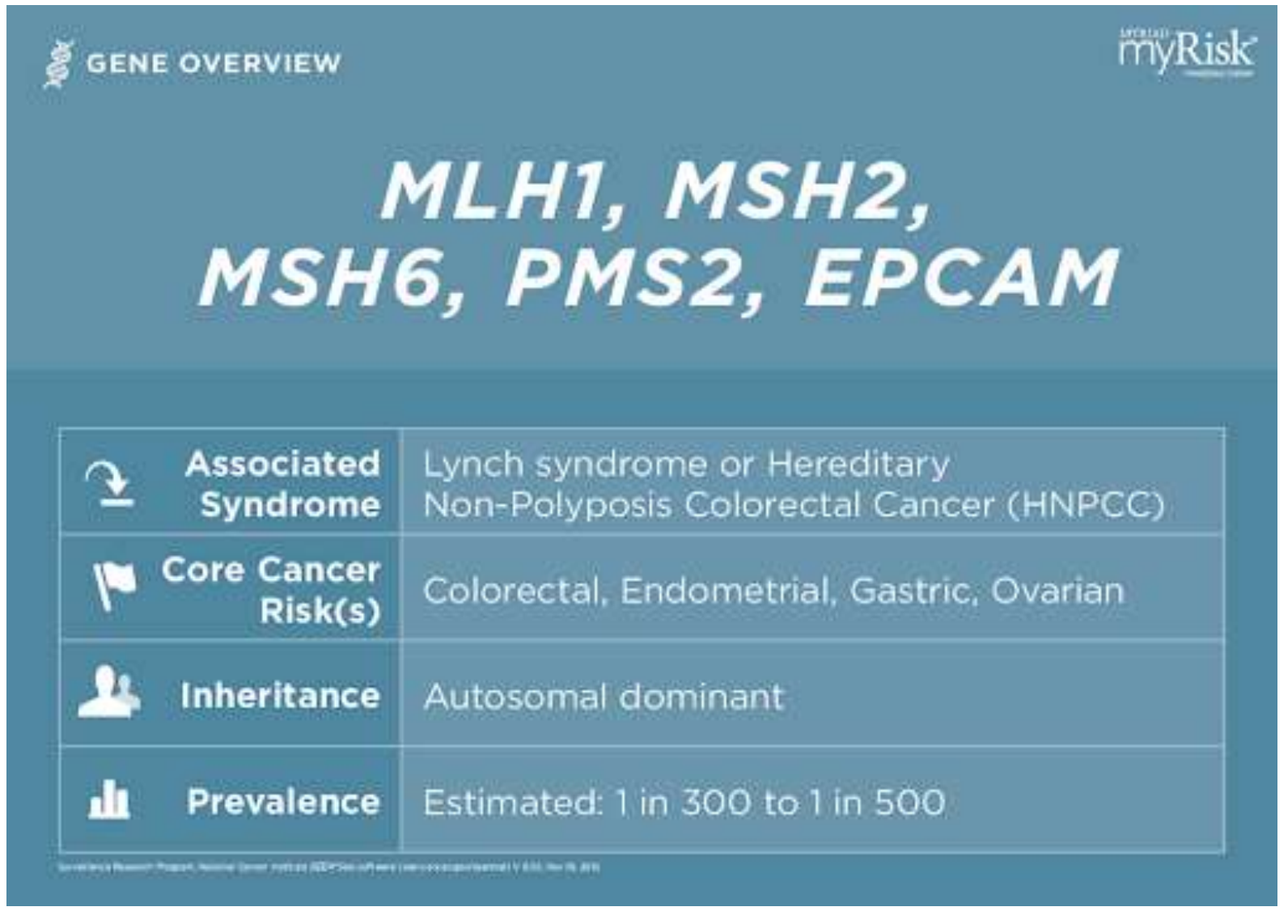
Heterozygotes for the most commonly mutated LS gene have an approximately 80% lifetime risk for development of cancer of the colon;

female heterozygotes have a somewhat smaller risk (approximately 70%) but also have an approximately 40% risk for endometrial cancer. i.e: endometrial cancer with family Hx of colon cancer → suspicion of LS in ddx.



* Genes linked to LS play a role in correction of mismatch repair. (MLH1, MSH2, MSH6, PMS2)
* If those genes are mutated → defective mismatch repair

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.

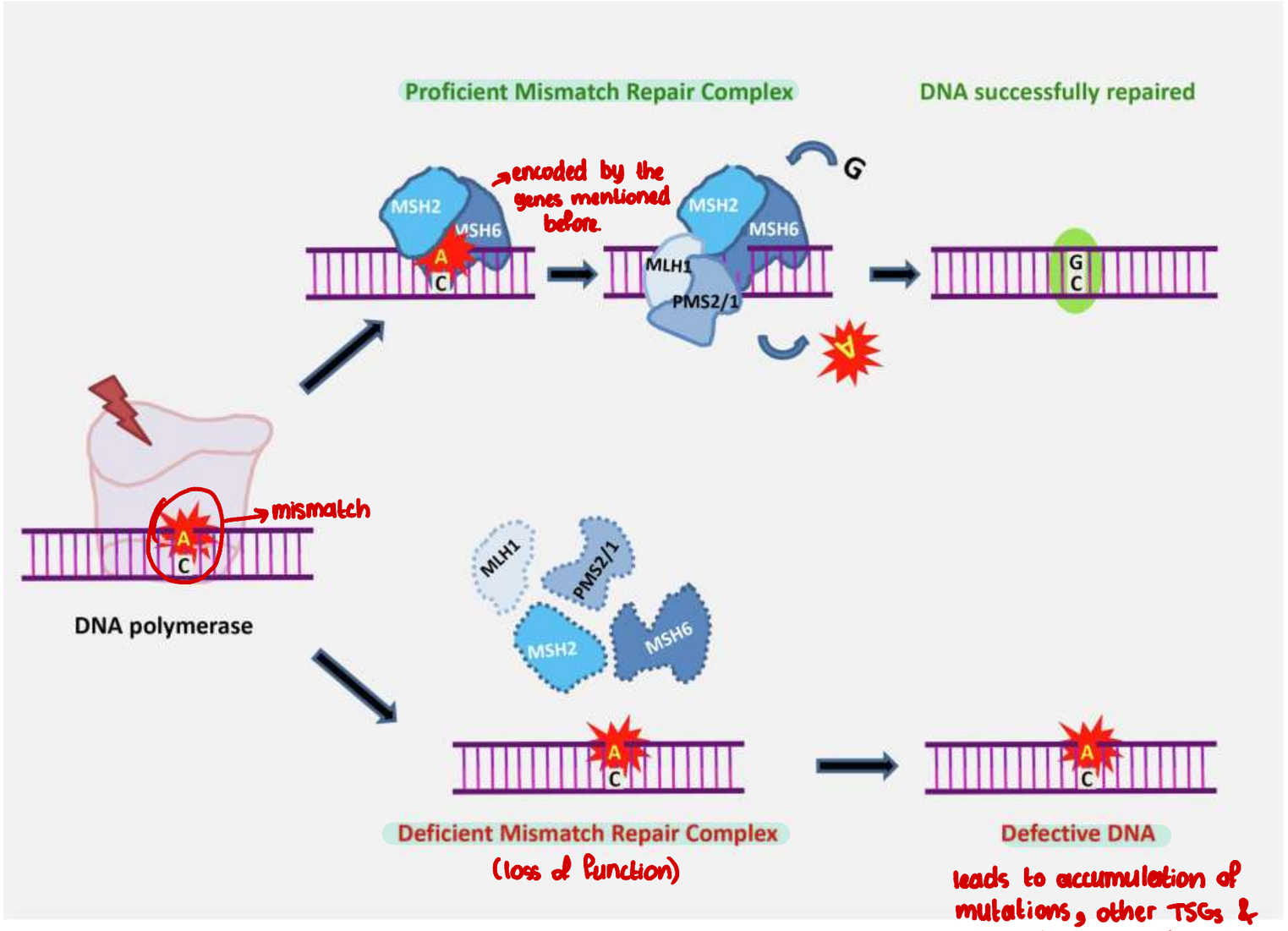


Although all four of these genes have been implicated in LS in different families, MLH1 and MSH2 are together responsible for the vast majority of LS, whereas the others have been found in only a few patients and are often associated with a lesser degree of mismatch repair deficiency and lower penetrance.

* MLH1 / MSH2 → higher risk.

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.



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

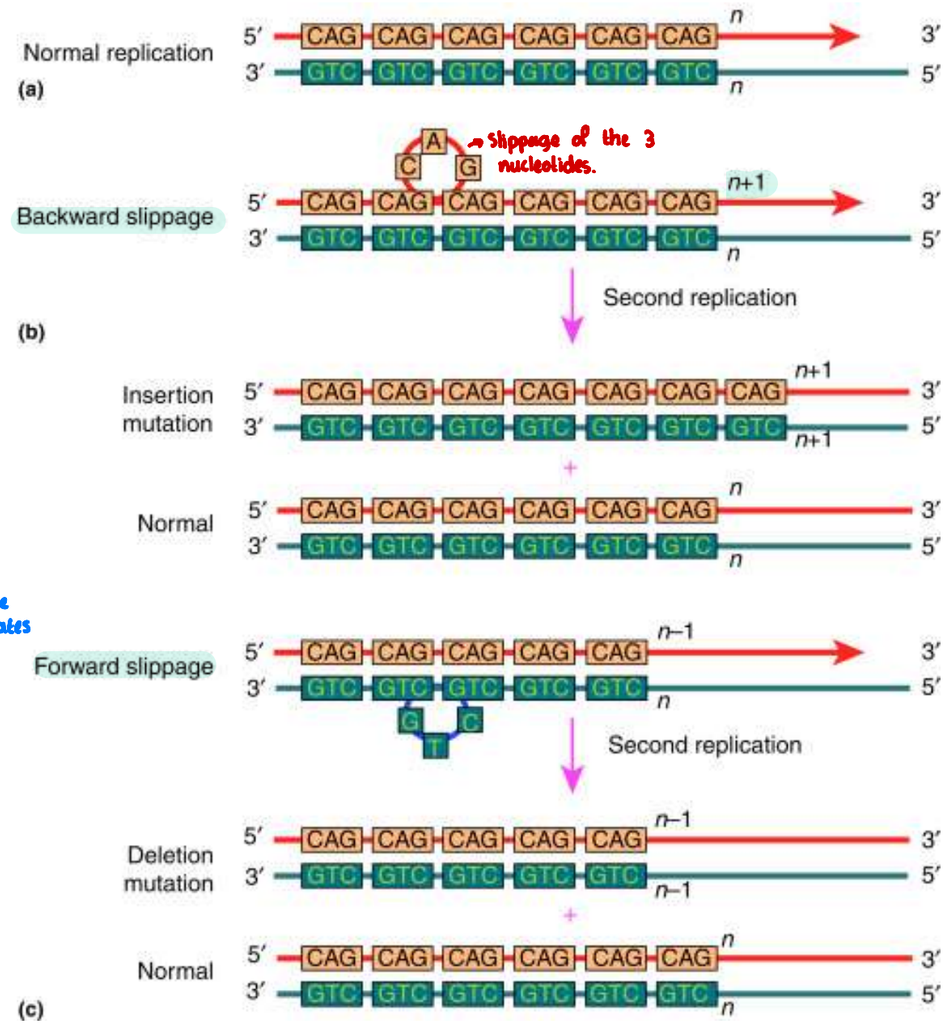
DNA polymerase finds more difficulty in replicating repeats w/o errors such as → homopolymers (i.e: AAAAA...)

→ microsatellites: a few nucleotides tandemly repeated (i.e: CAGCAGCAGCAG...)

} those regions have higher mutation rates than normal

Using the template strand, DNA polymerase might make errors in the newly synthesized strand resulting in different # of repeats (+/-) due to deletion OR insertion OR slippage. (NOT corrected in LS due to impaired mismatch repair)

One of the tests for LS is to examine a repeat-rich region to check if repeats are replicated correctly or not (microsatellite instability)



* PCR amplification for comparison between normal & tumor tissue.

* those markers are for repeat-rich regions.

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

* these could also be many other mutations in coding genes like TSGs & oncogenes.

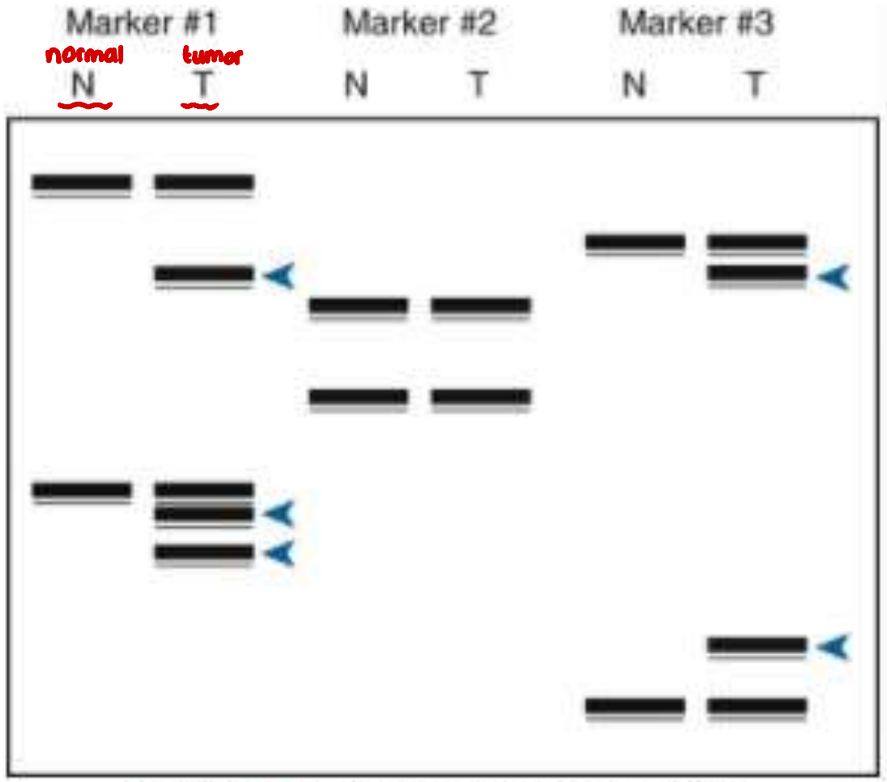


FIGURE 15-9 Gel electrophoresis of three different microsatellite polymorphic markers in normal (N) and tumor (T) samples from a patient with a mutation in *MSH2* and microsatellite instability. Although marker #2 shows no difference between normal and tumor tissues, genotyping at markers #1 and #3 reveals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

↓

due to expansion / shrinkage during replication of some regions that are harder to amplify (repeat-rich) AND it is NOT corrected.

↓

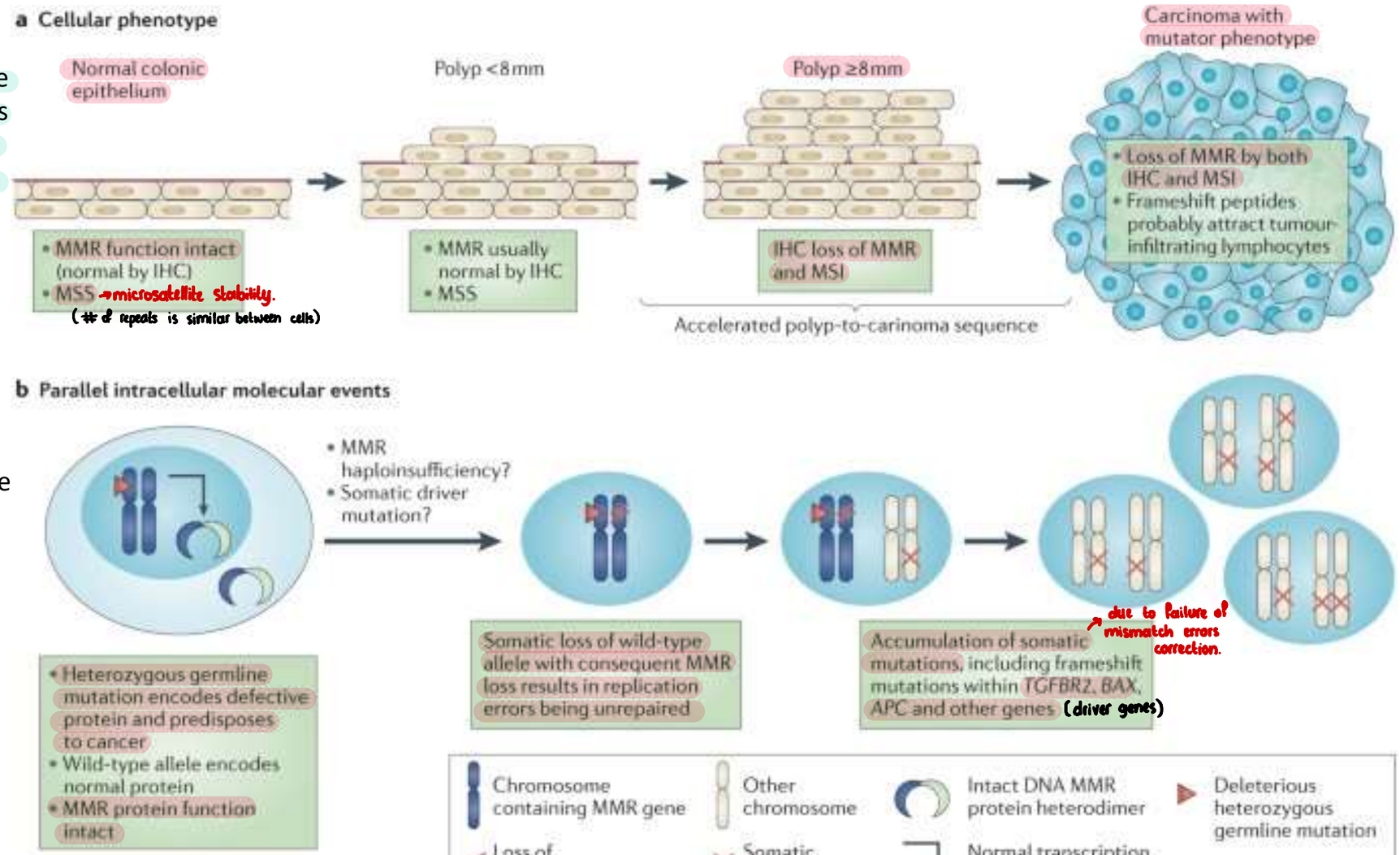
results in different sizes of those regions. (even different between 2 groups of cells within the same tumor)

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

(figure on the rt): Binding of TGF-β to TGFBR2 results in phosphorylation of → ERK ptn. / → SMAD 2/3 ptn → SMAD complex (P-SMAD2/3 + SMAD4) → migrate into the nucleus → inhibit expression of MYC (proto-oncogene (induces cell cycle & division)) / induces expression of P21 (TSG) → inhibition of cell cycle

* When TGFBR2 is mutated, it loses phosphorylation ability (normal response upon TGF-β binding), consequently SMADs will NOT inhibit MYC expression & will NOT induce P21 expression. Therefore cell cycle is NOT inhibited.

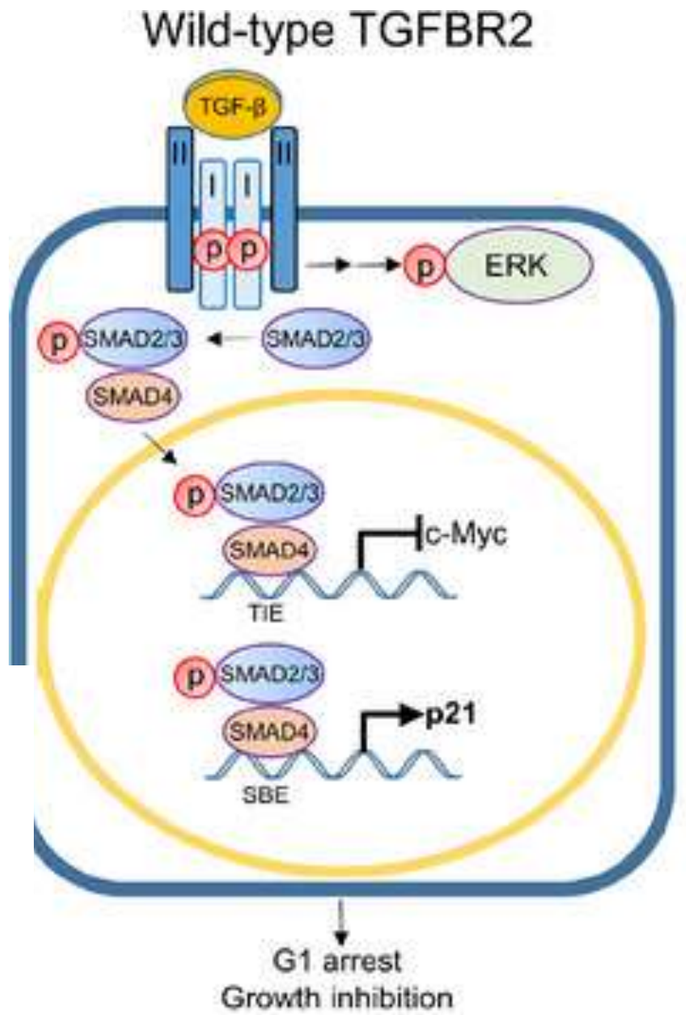
↳ found on the cell membrane.

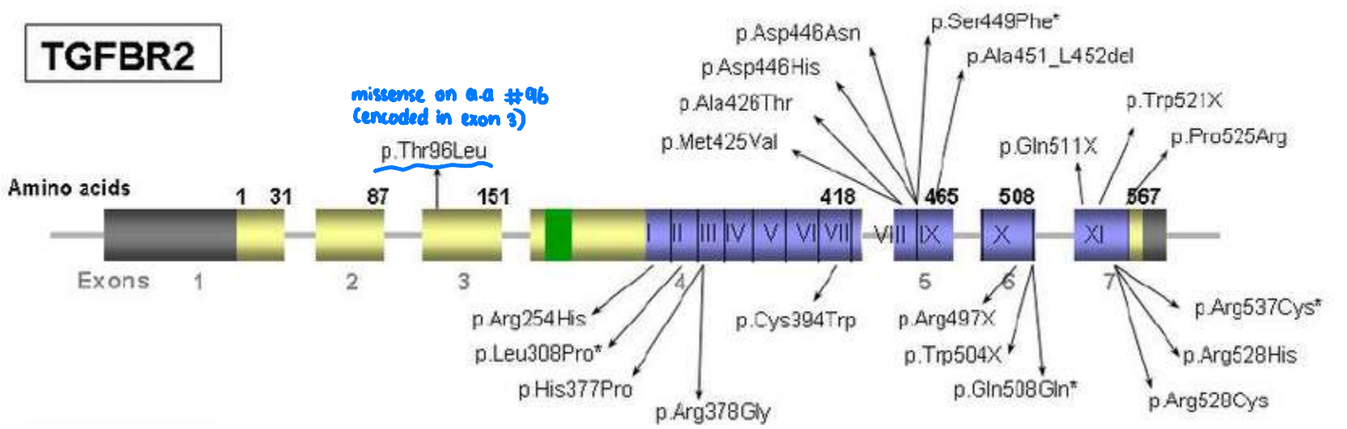
TGFBR2 encodes transforming growth factor β receptor II, a serine-threonine kinase that inhibits intestinal cell division.

TGFBR2 , is particularly vulnerable to mutation when mismatch repair proteins are lost (after the 2nd hit) 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.





known mutations to cause loss of function of TGFBR2
i.e: missense, premature stop codon, splice site ...