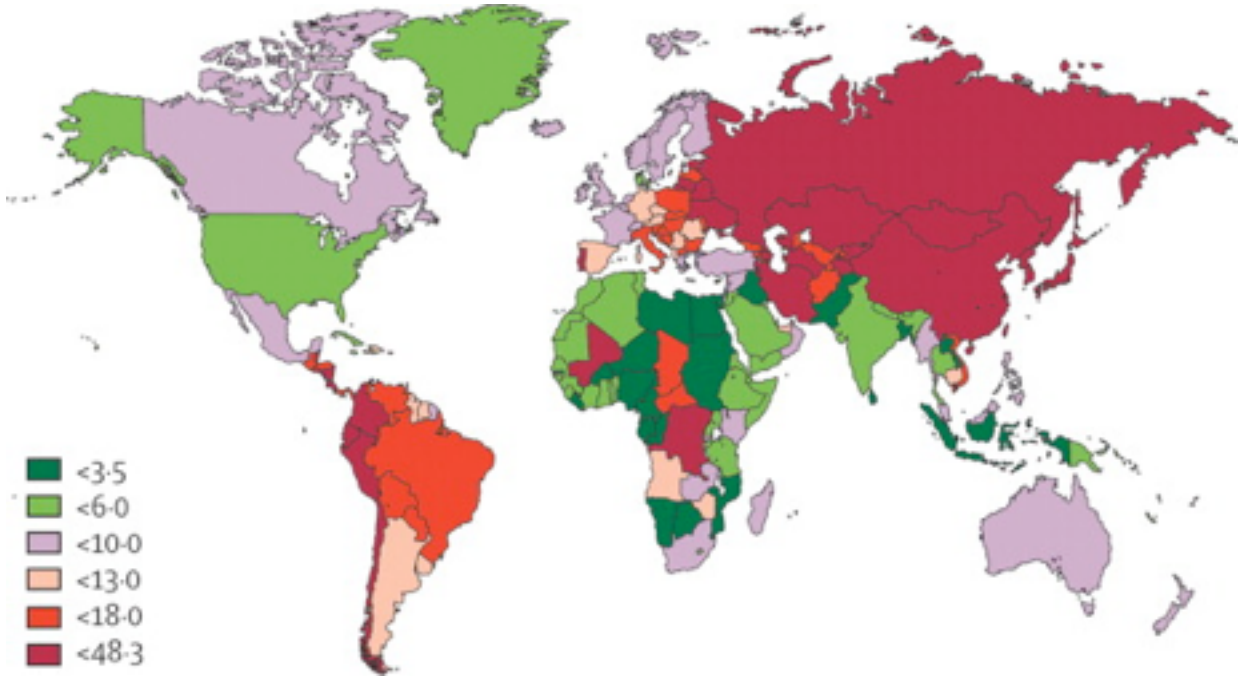
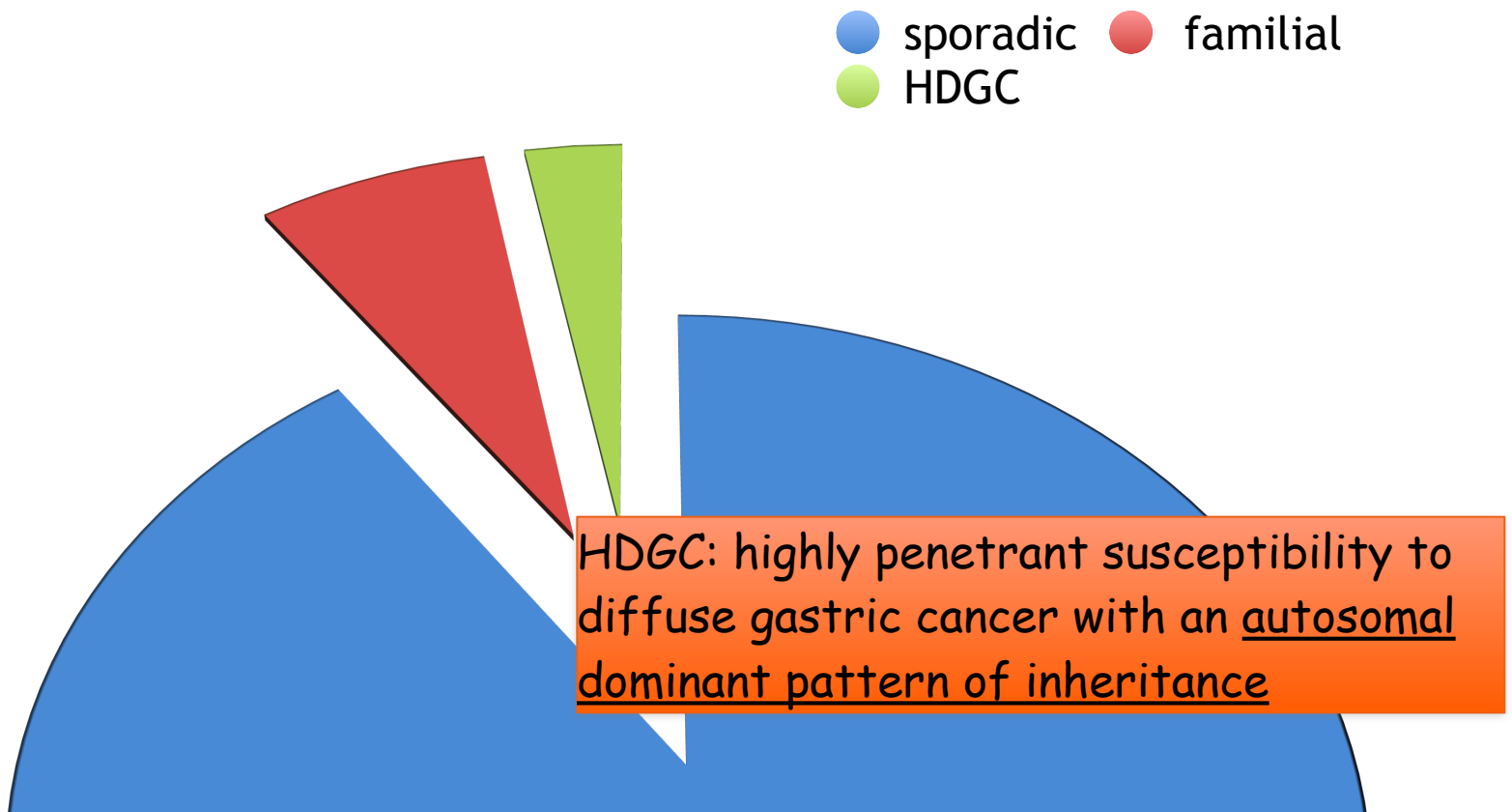
Gastric Cancer Incidence

- G.C. is still the second most common cause of cancer-related death in the world
- Incidence is decreasing in old patients and is stable in young patients and cases with familial clustering



Italy: moderate to high risk area

Gastric cancer cases



HDGC: highly penetrant susceptibility to diffuse gastric cancer with an autosomal dominant pattern of inheritance

G.C. cases

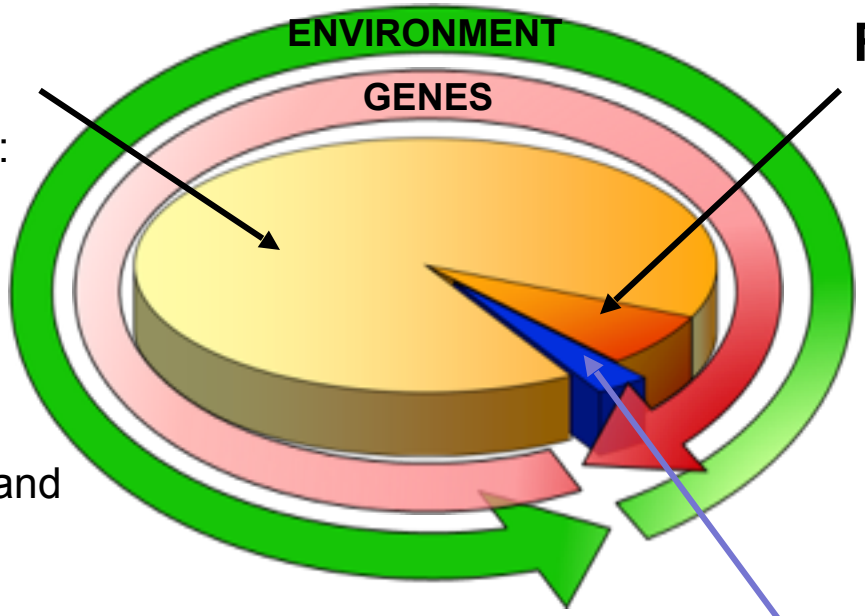
Sporadic (90%)

Intestinal-type prevailing:

<u>old patients</u>

high risk areas

risk factors including *H. pylori,* alcohol intake and smoking



Familial (10%)

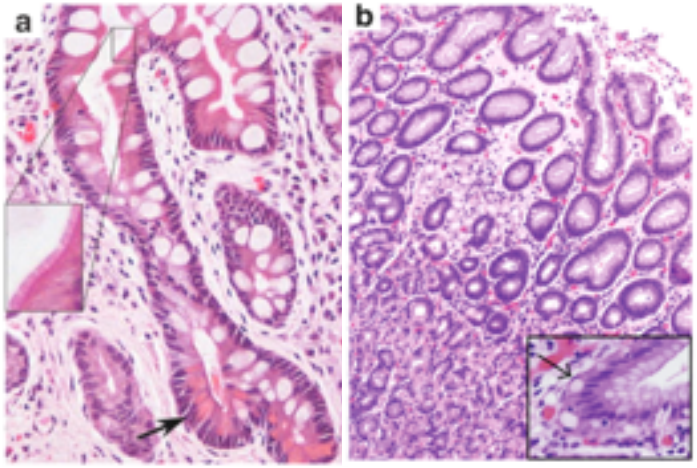
Diffuse-type prevailing:

<u>young patients</u>

homogeneous geographic distribution

genetic factors

HDGC (1-3%)



Sporadic GC

1. GC is the end result of a multifactorial, multigenetic and multistage process

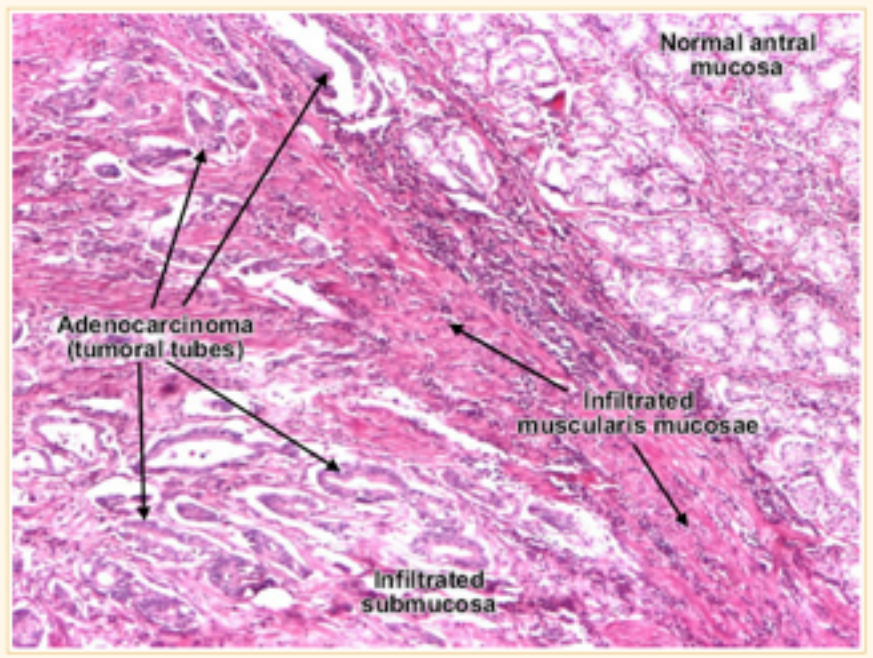
2. arious environmental exposures such as Helicobacter pylori infection, high salt diet, inadequate vitamin C uptake and smoking, have been identified as risk factors

Sporadic GC

1. Gastric cancer (GC) remains a leading cause of cancer-related deaths worldwide, even though a decrease in its incidence and mortality rate has been observed in recent decades

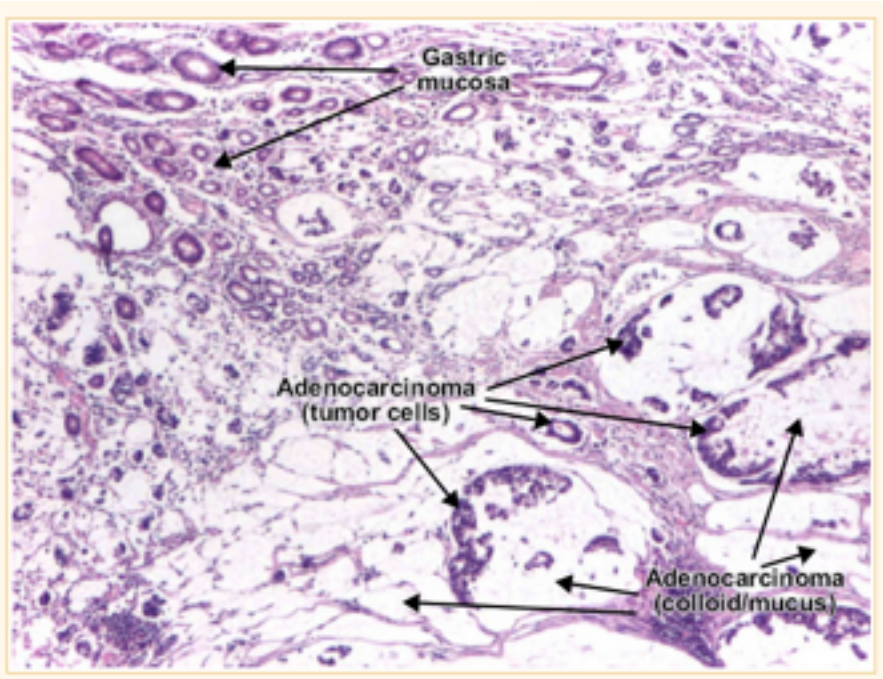
2. Although the incidence of intestinal GC has declined gradually, the incidence of diffuse gastric cancer (DGC) has remained stable.

95% of G.C. are adenocarcinomas which can be classified into intestinal and diffuse types



Gastric adenocarcinoma, intestinal type.

Tumor cells describe irregular tubular structures, with stratification, multiple lumens surrounded by a reduced stroma ("back to back" aspect). The tumor invades the gastric wall, infiltrating the muscularis mucosae, the submucosa and thence the muscularis propria. Often it associates intestinal metaplasia in adjacent mucosa. Depending on glandular architecture, cellular pleomorphism and mucosecretion, adenocarcinoma may present 3 degrees of differentiation : well (photo), moderate and poorly differentiate.

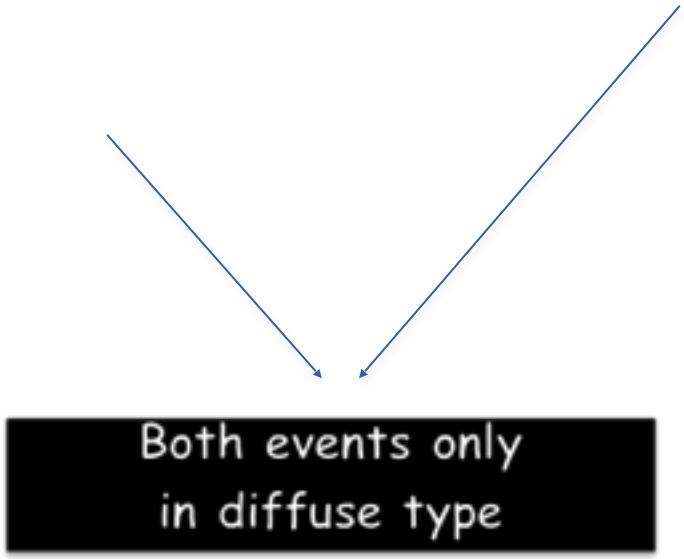


Gastric adenocarcinoma, diffuse (infiltrative) type.

Tumor cells are discohesive and secrete mucus which is delivered in the interstitium producing large pools of mucus/colloid (optically "empty" spaces) - mucinous (colloid) adenocarcinoma, poorly differentiated (Lauren classification).
If the mucus remains inside the tumor cell, it pushes the nucleus against the cell membrane - "signet-ring cell".

E-cadherin (*CDH1* gene) - somatic lesions

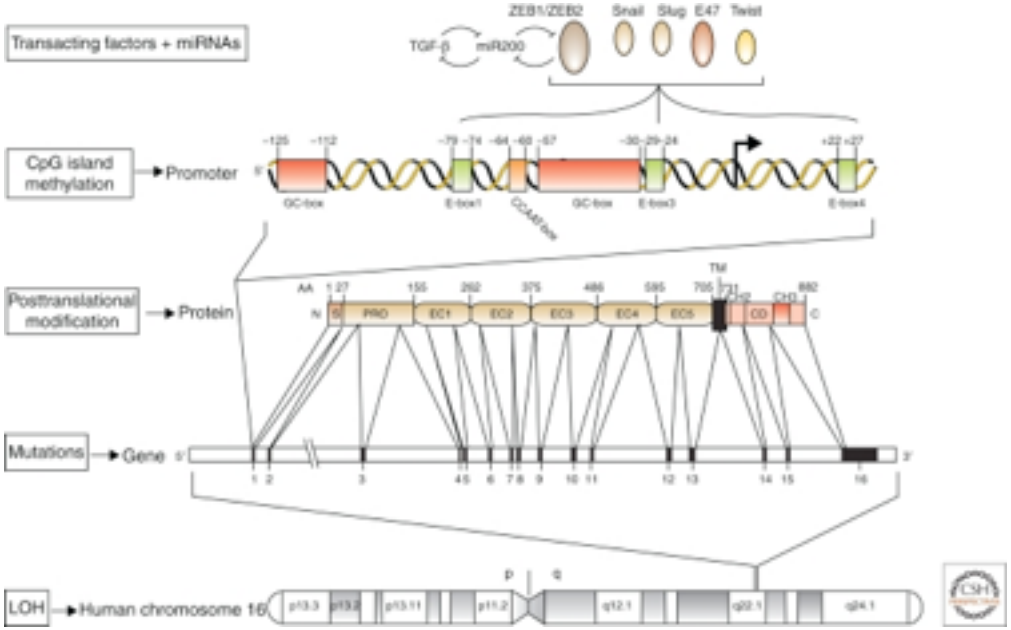
- Mutations in 50% of cases

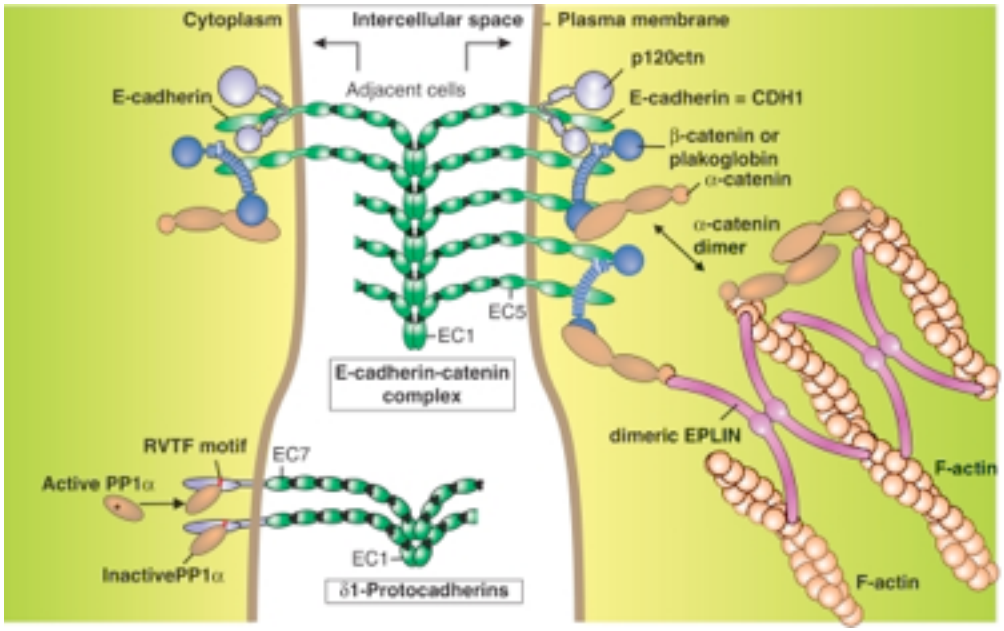


- Loss of the expression of one allele in 40% of cases
- Loss of the expression of one allele is also present in 40% of intestinal tumors

Becker and Hofler; J Natl Cancer Inst 1995

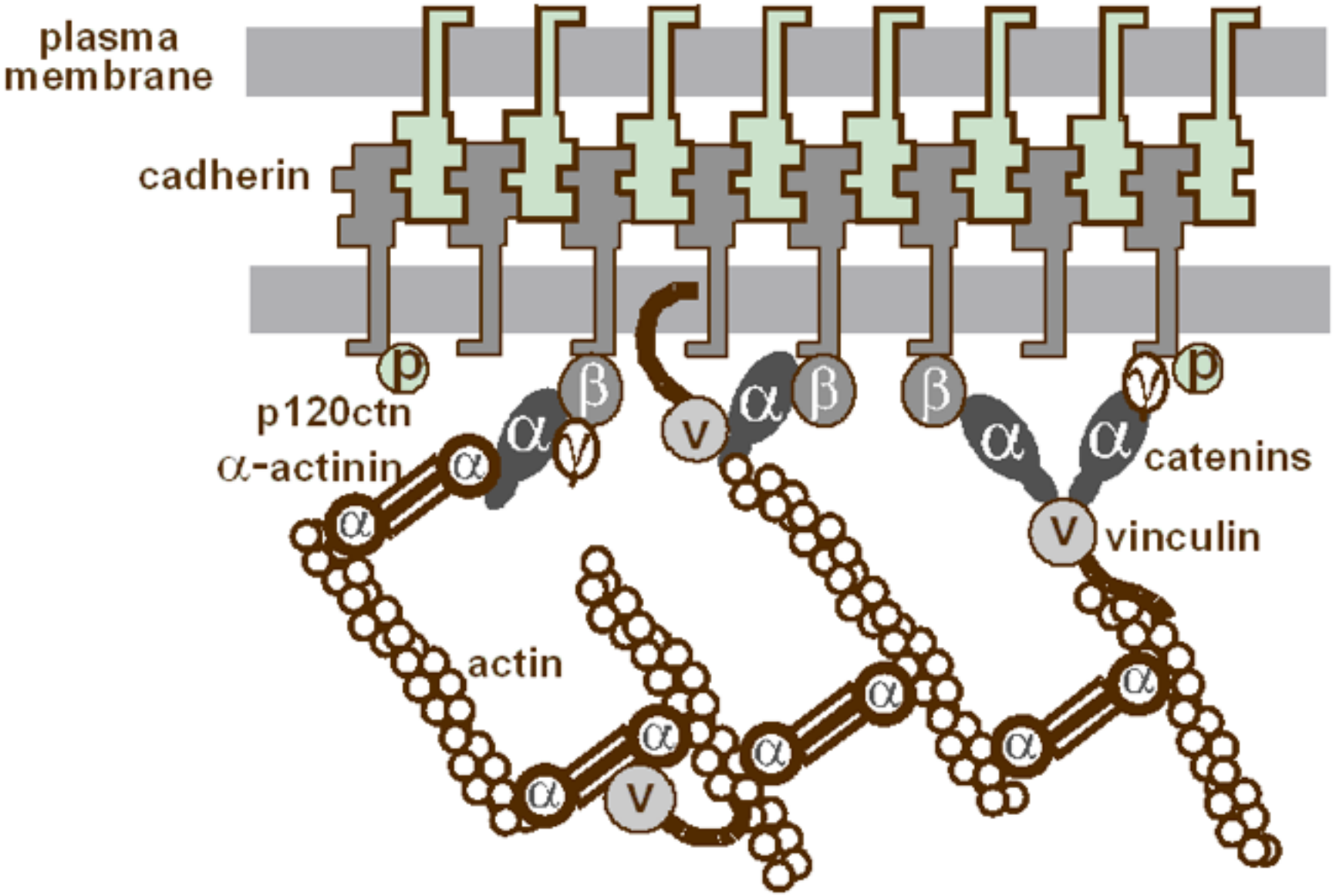
CDH1 gene

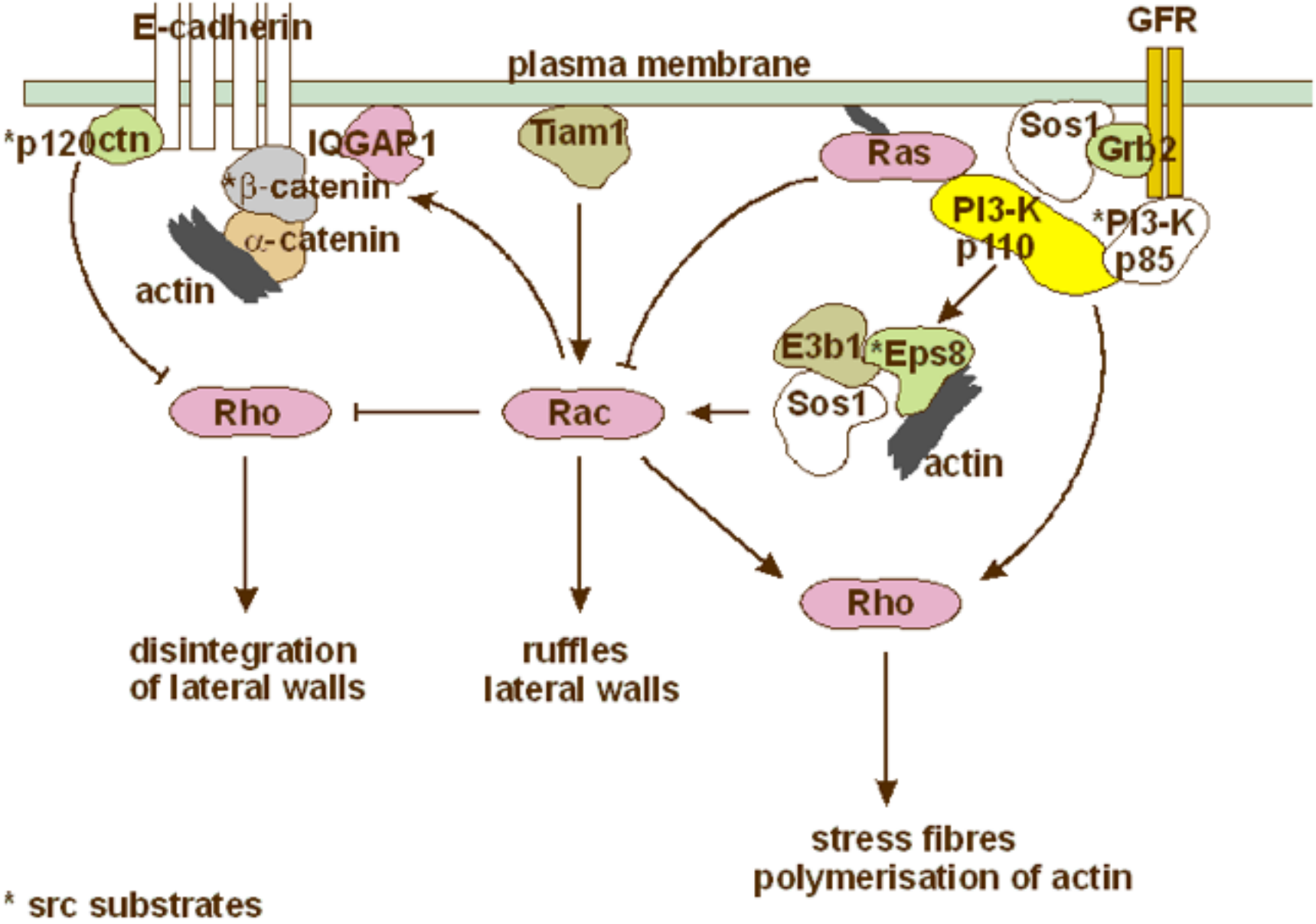




E-cadherin protein

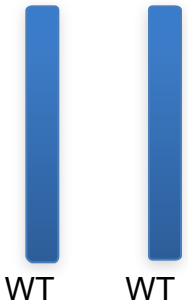
ZONULA ADHERENS

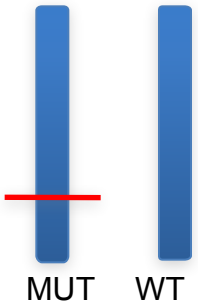


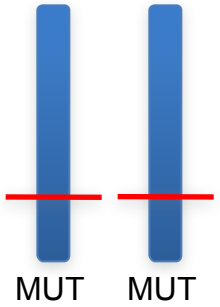

E-cadherin
plasma membrane
GFR
*p120ctn
IQGAP1
*β-catenin
α-catenin
actin
Tiam1
Ras
Sos1
Grb2
PI3-K p110
*PI3-K p85
E3b1
*Eps8
Sos1
actin
Rho
Rac
Rho
disintegration of lateral walls
ruffles lateral walls
stress fibres polymerisation of actin
* src substrates

CDH1 loss of function and E-cadherin expression

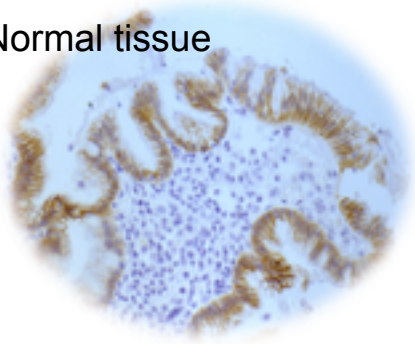
CDH1 is a t.s.g. and follows the “two hit” model



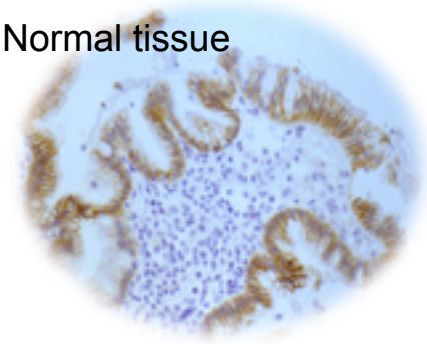


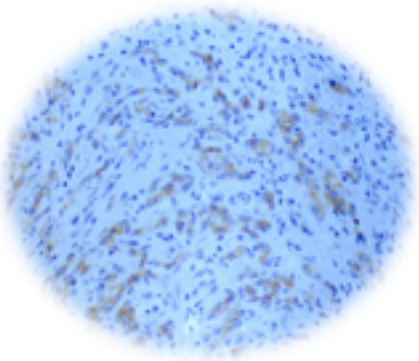


Normal tissue

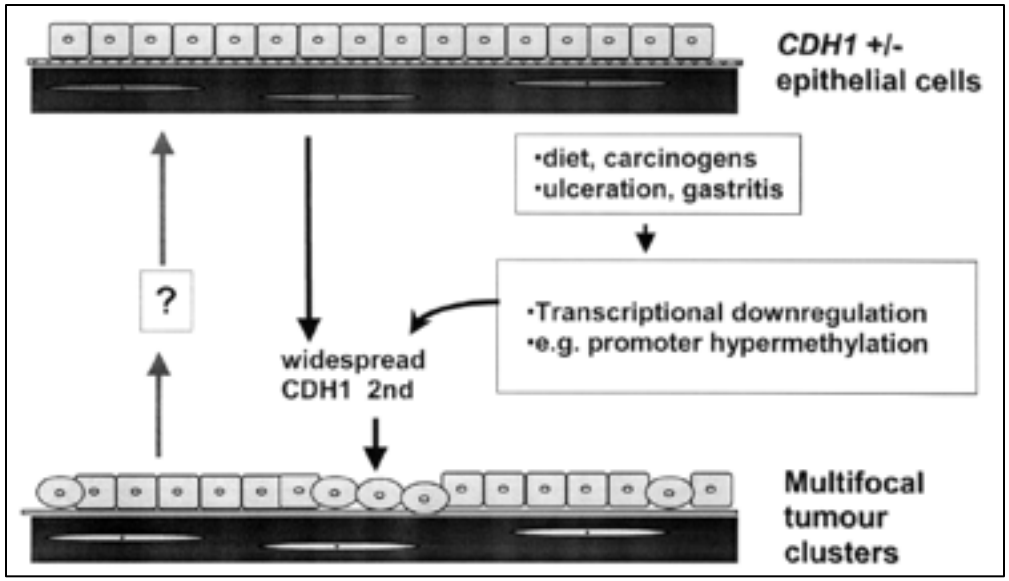


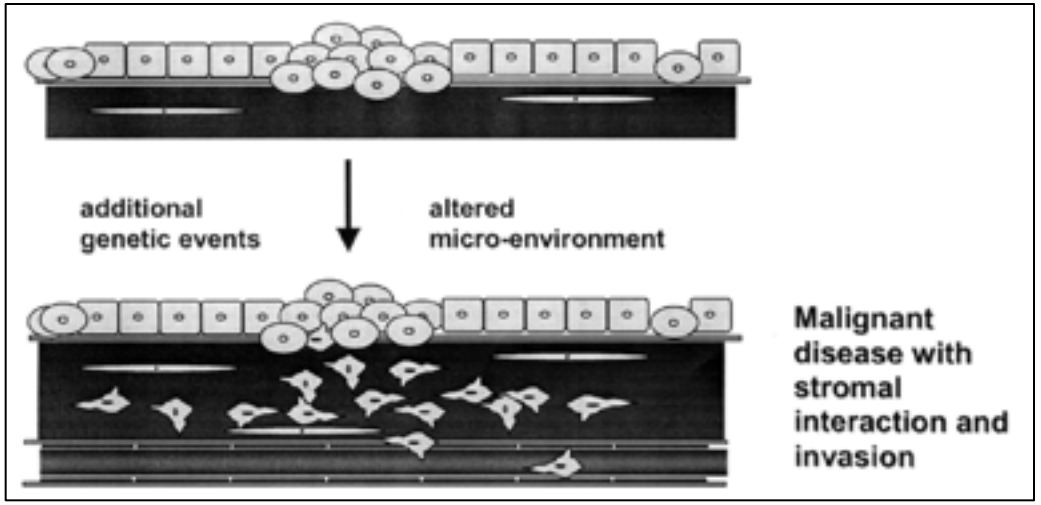
Normal tissue





Cancer tissue in both hereditary and sporadic DGC and LBC cases





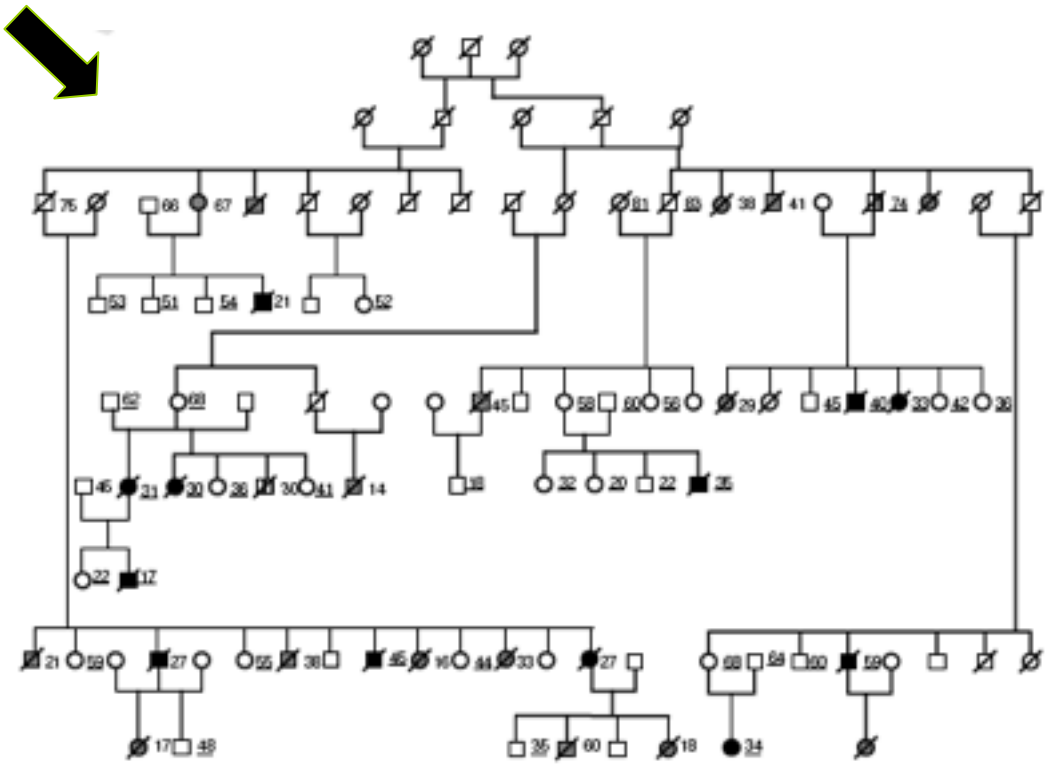
(From: Graziano F, Humar B, Guilford P.; Ann Oncol. 2003)

E-cadherin (*CDH1*) germline mutations in familial gastric cancer

(Guilford et al. Nature 1998)

CDH1 mutations in 3 Maori kindred

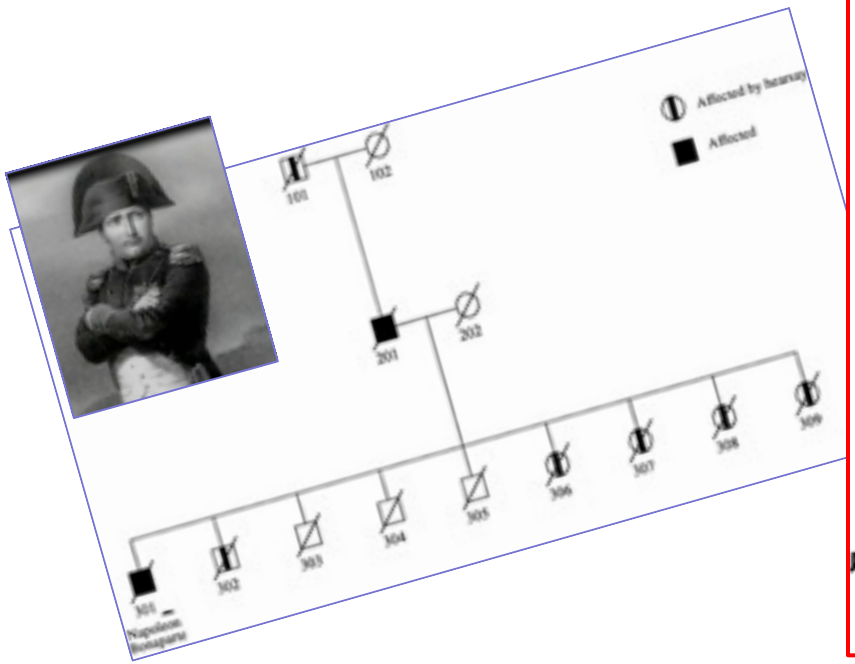
with early-onset diffuse-type gastric cancer

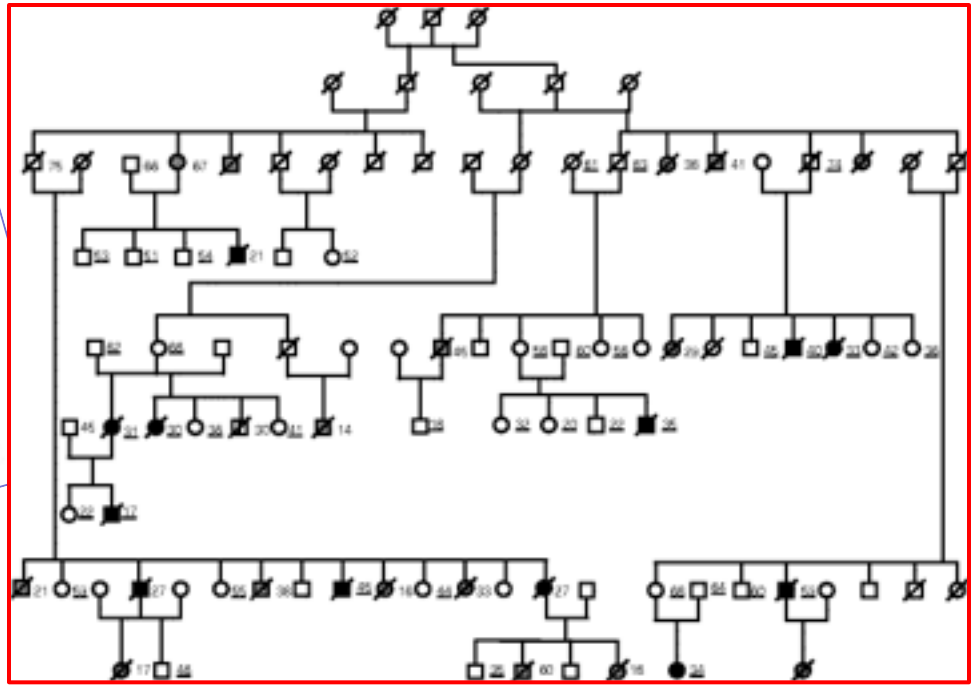


HDGC syndrome

- **Autosomal dominant cancer-susceptibility syndrome**
- **Gastric cancer of diffuse histotype (and Lobular Breast Cancer)**
- **Average age of onset: 38 years**
- **Associated with inactivating mutation of *CDH1* gene**
- **High penetrance (70%)**

(Guilford et al., 1998)





CDH1 follows the "two hit" model

In hereditary diffuse gastric cancer one allele is **constitutively mutated**

The inherited wild-type allele is inactivated in tumor cells by **a somatic event**

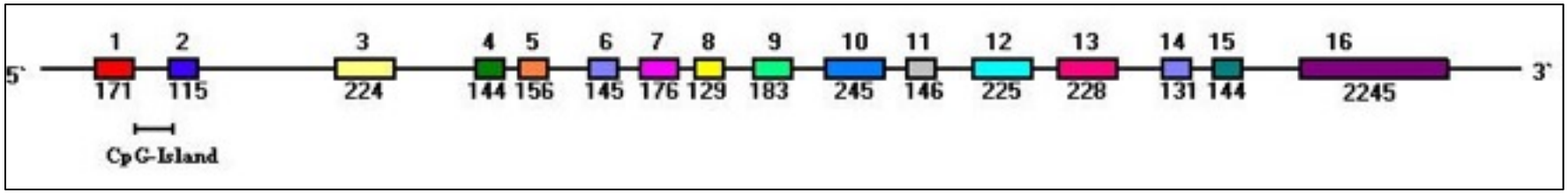
Methylation of the CDH1 promoter is the second hit (somatic mutations, and intragenic deletions also found)

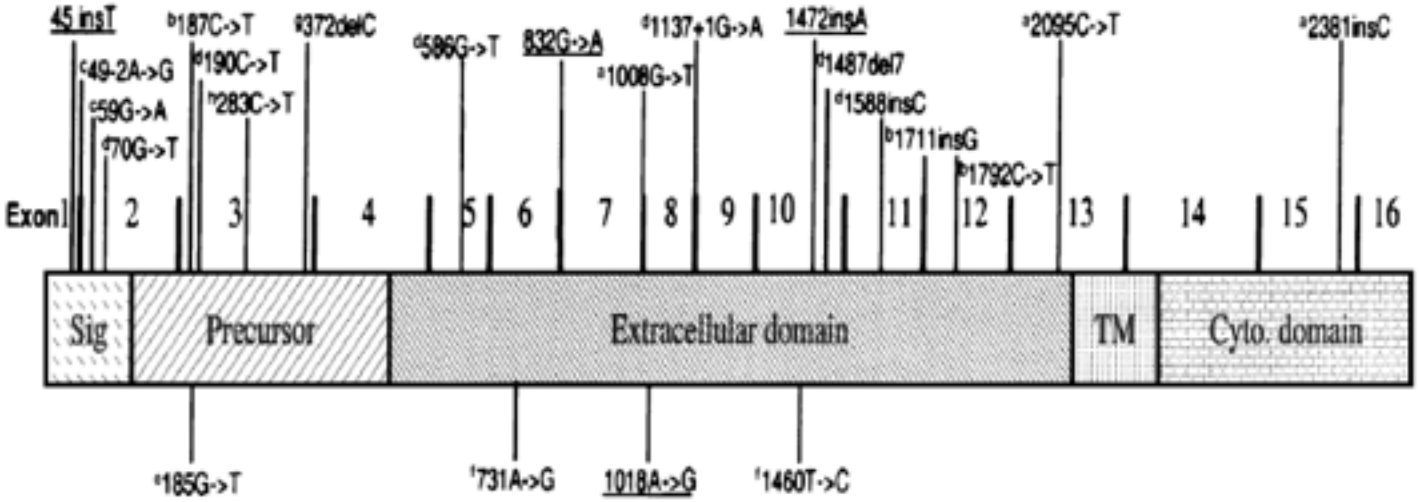
(Grady et al. Nat Genet 2000 ; Oliveira et al. Oncogene 2004)

…CDH1 identified mutations

GENE SEQUENCING

- the great majority (about 80%): truncating mutations (frameshift, nonsense, splicing)
- about 20%: missense mutations





…CDH1 identified mutations

DELETIONS

6.5%: recently identified by MLPA in "mutation-negative" HDGC families

3.8%: overall freq. (only in countries with low incidence of G.C. accounting for 9% of all CDH1 alterations in HDGC families)

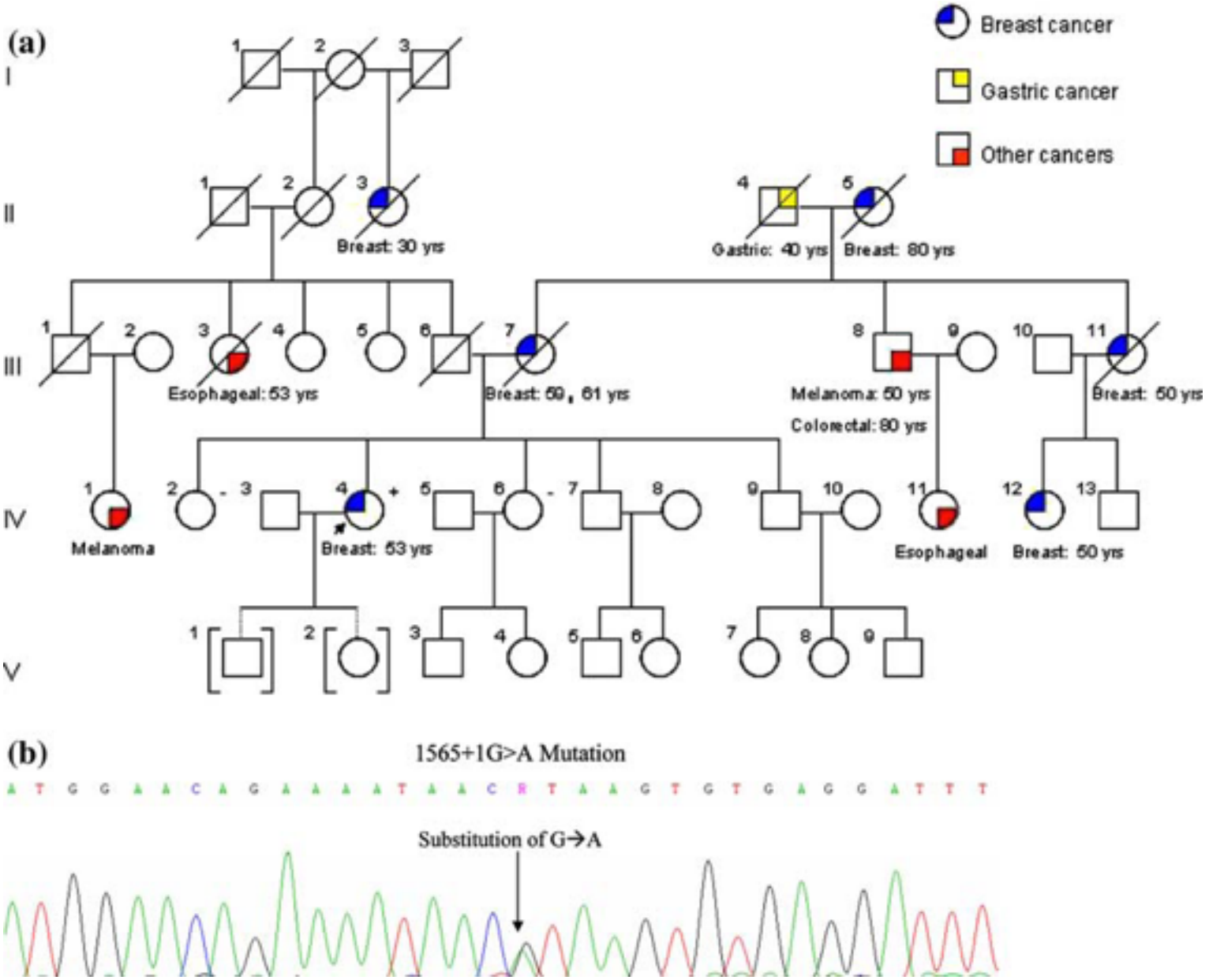
(Oliveira et al. Hum Mol Genet 2009)

HDGC syndrome: definition criteria

- The predictions were validated by enlarging the number of families screened for CDH1 mutations: **30-40%** of the HDGC families were found to harbor pathogenic mutations

- ……..CDH1 pathogenic mutations also found in about **40%** families with: 2 or more G.C. cases in the same family, with at least 1 diffuse G.C. diagnosed before the age of 50 (Brooks-Wilson et al. J Med Genet 2004)

Additional or exclusive Lobular BC



Lobular breast cancer

Selection criteria:

- women with documented invasive LBC or mixed ductal breast cancer and LBC at any age
- no reported relatives with gastric tumours and
  - either family history with >=2 cases of breast cancer in first or second degree relatives in the maternal and paternal lineage, including third degree relatives in the paternal lineage;
  - or LBC or mixed breast cancer diagnosed in the proband before 45 years of age independent of family history

Results:

- CDH1 germline mutations: 1 out 23 cases
- Germline mutations can be associated with invasive LBC in the absence of diffuse G.C.

(Masciari et al. J Mol Med 2008)

….cumulative risk in CDH1 mutation carriers

- estimated cumulative risk of gastric cancer by age 80 years: 67% for men and 83% for women

- for women:
  - –cumulative risk of breast cancer 39%
  - –combined risk of gastric cancer and breast cancer 90% by age 80 years

(Pharoah et al. Gastroenterology 2001)

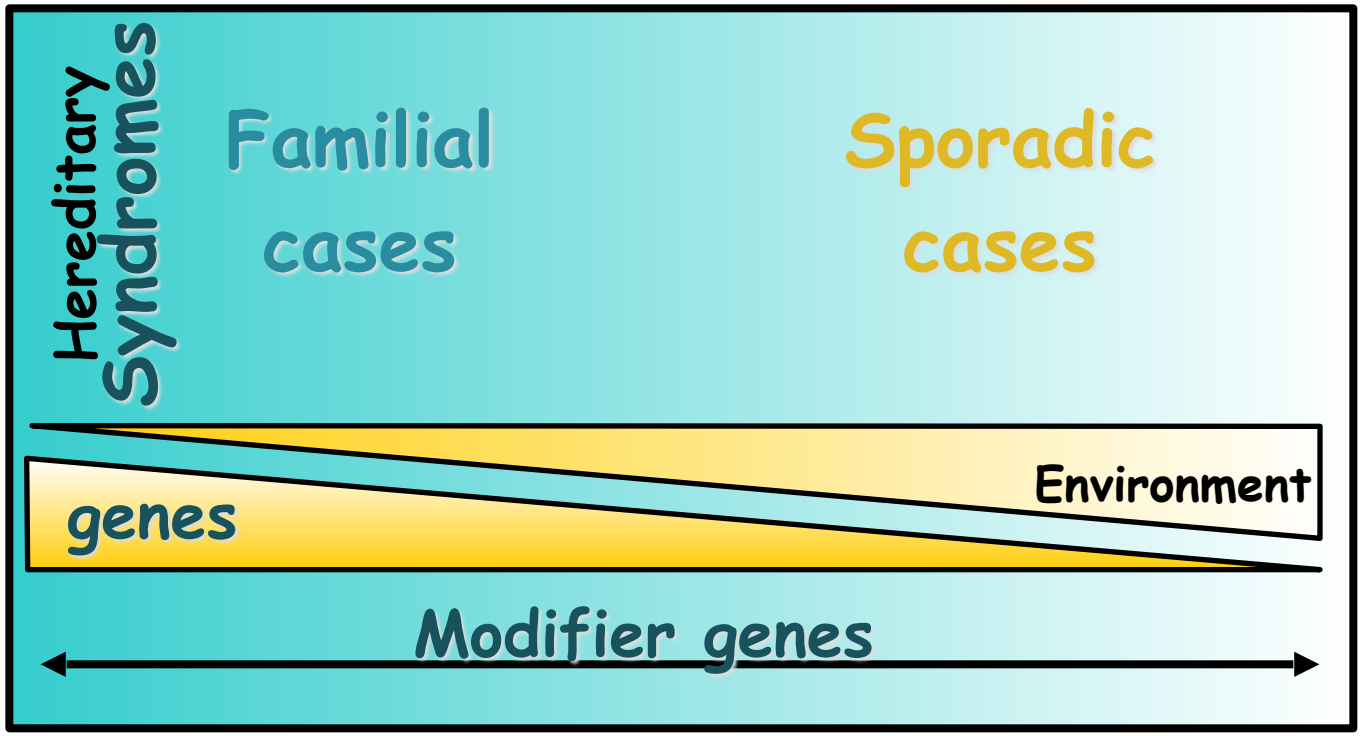
HEREDITARY GC: a polygenic syndrome

- **CDH1**

  Lobular breast carcinomas, colorectal carcinomas, and prostatic carcinomas have been documented in mutant gene carriers

- **Other genes**
  - MMR genes (gastric cancer belongs to the tumor spectrum of HNPCC)
  - p53 (mutations have been found in cancer families with different tumor types including gastric cancer)
  - Other candidates ???

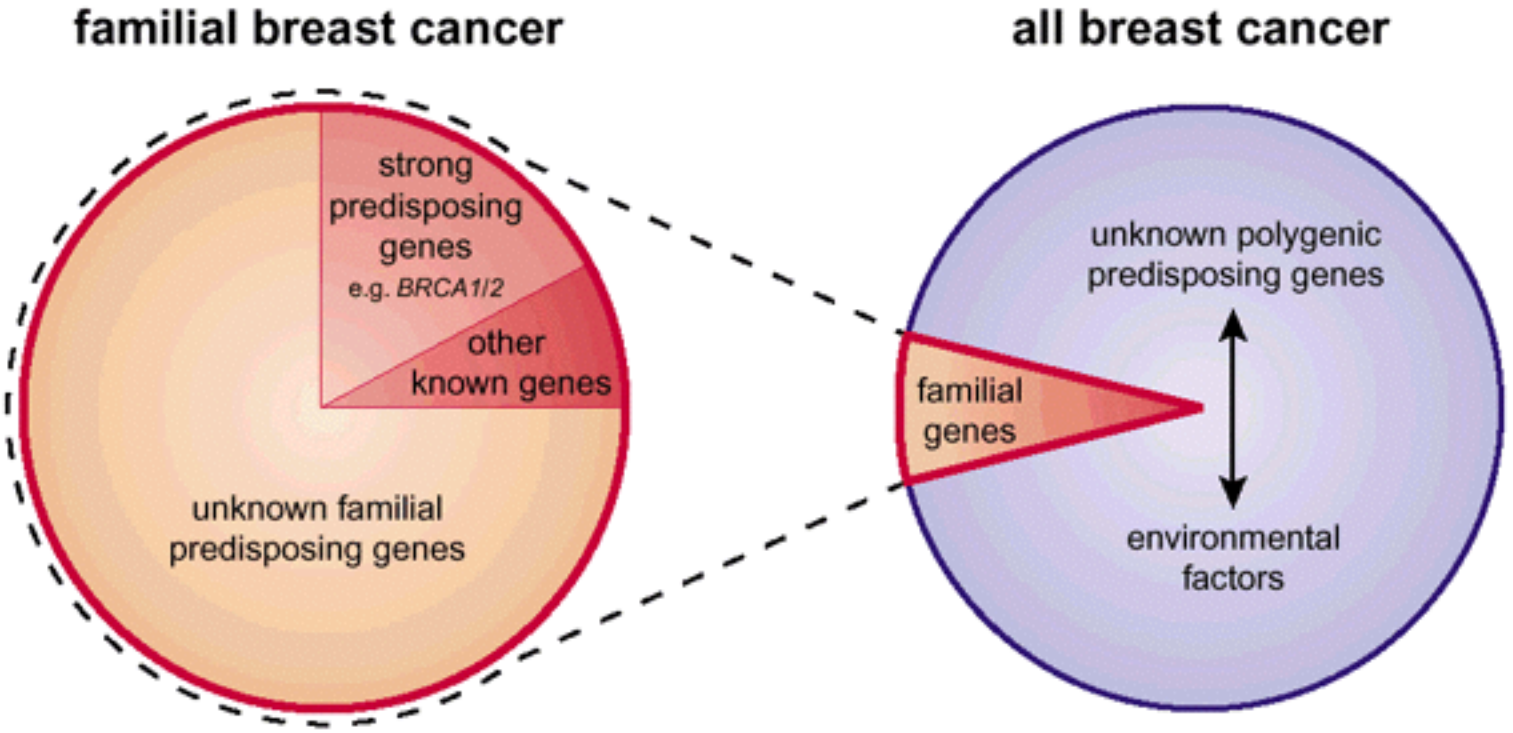
Breast cancer



Breast cases are different in respect to colorectal cancers:

few hereditary cancer genes and more low penetrant alleles

Breast cancer



Breast cancer

Breast cancer risk is 1/12 women

Hereditary cases are 5% of the total breast tumours

High penetrant genes:

BRCA1, BRCA2

PTEN

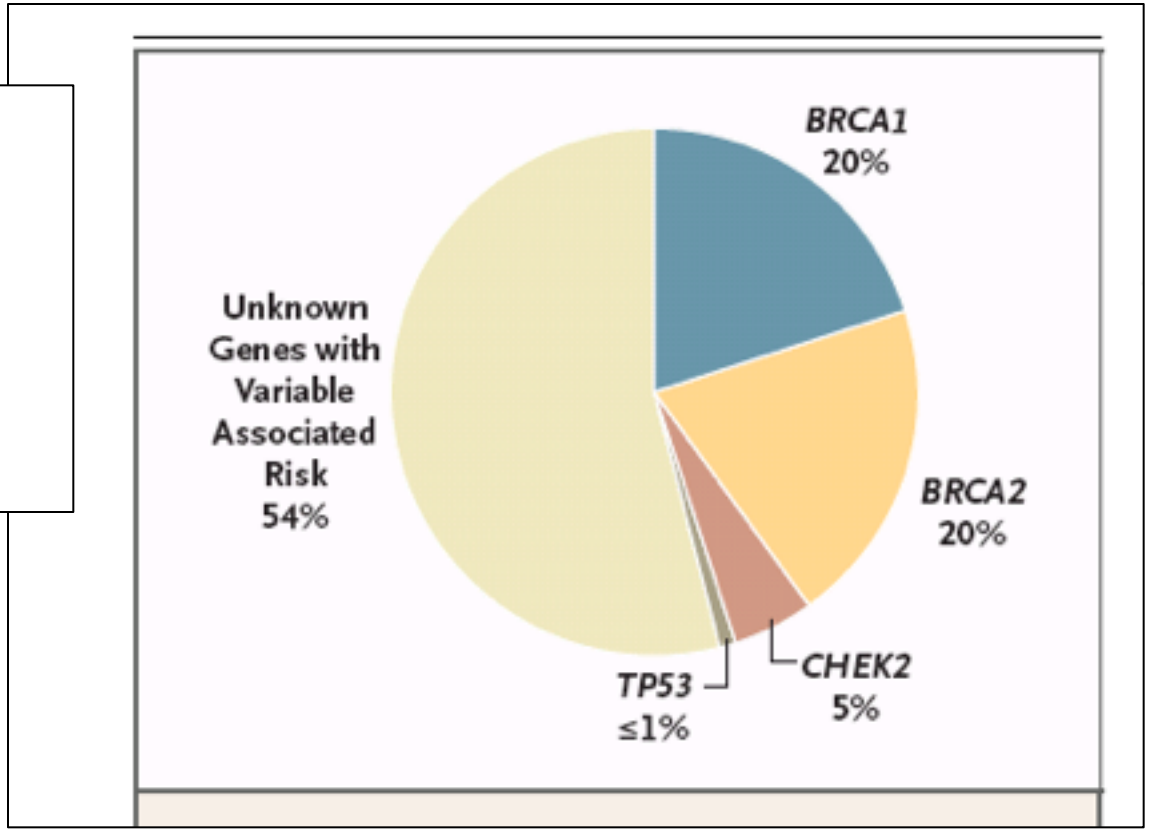
P53

LKB1/STK11

CDH1

BRCA1/2 genes account for only a subset of the hereditary breast cancers

High, but variable penetrance



Polygenic syndrome

Low penetrant alleles: CHECK2

Breast cancer

BRCA1/BRCA2

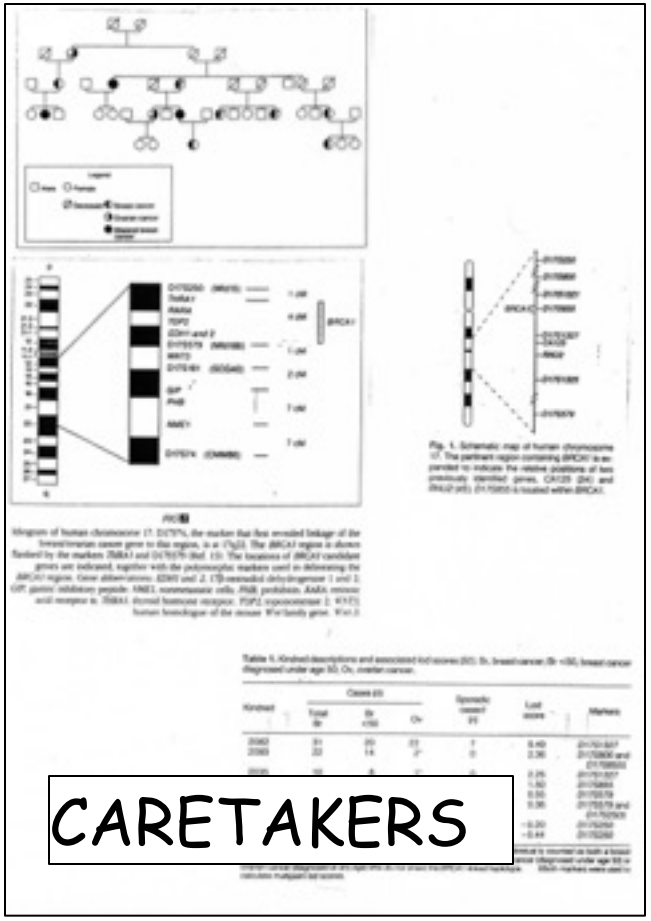
•2-3% of all breast/ovarian cases

• both identified with linkage analysis

BRCA1 : **17q21, 100Kb, 22 exons**

BRCA2 : **13q12, 70Kb, 27 exons**

BRACA2 also in linkage with male breast cancer



CARETAKERS

BRCA1 : DNA repair, chromatic remodelling and cell cycle check control

BRCA2 : DNA repair (DSB) during homologous recombination

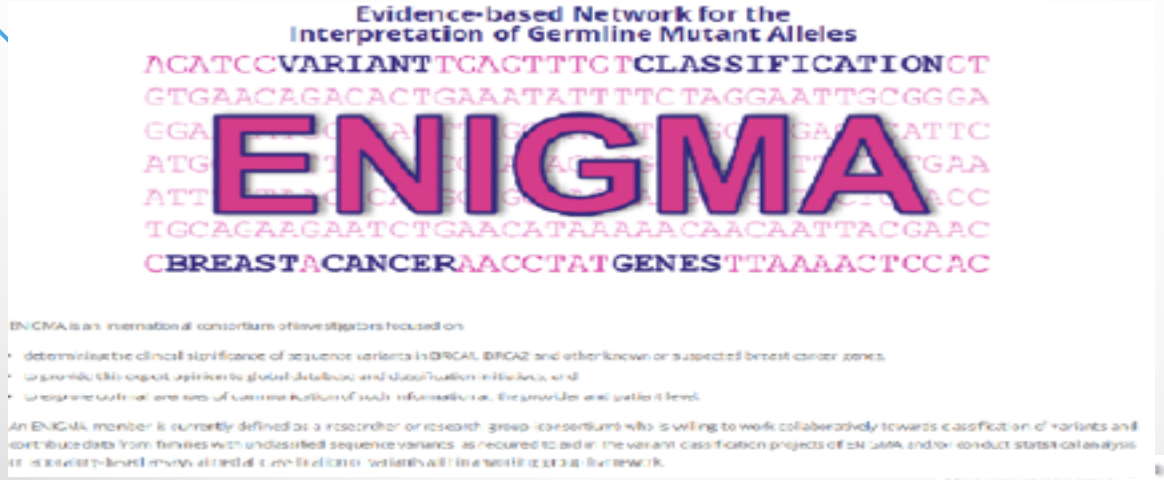
• cancer risk at 70 years

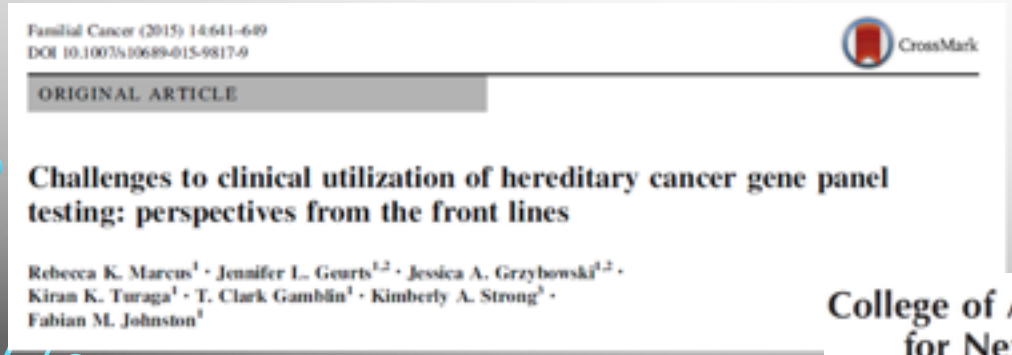
BRCA1 : **46% breast ; 39% ovarian**

BRAC2 : **43% breast ; 27% ovarian**

Genotype-phenotype : **mutations in the central domain are associated with a more aggressive phenotype**

Very frequent SNPs; in addition to a major variant, SNPs may influence the phenotype




Familial Cancer (2015) 14:641–649
DOI 10.1007/s10689-015-9817-9

ORIGINAL ARTICLE

Challenges to clinical utilization of hereditary cancer gene panel testing: perspectives from the front lines

Rebecca K. Marcus[1] · Jennifer L. Geurts[1,2] · Jessica A. Grzybowski[1,2] · Kiran K. Turaga[1] · T. Clark Gamblin[1] · Kimberly A. Strong[3] · Fabian M. Johnston[3]

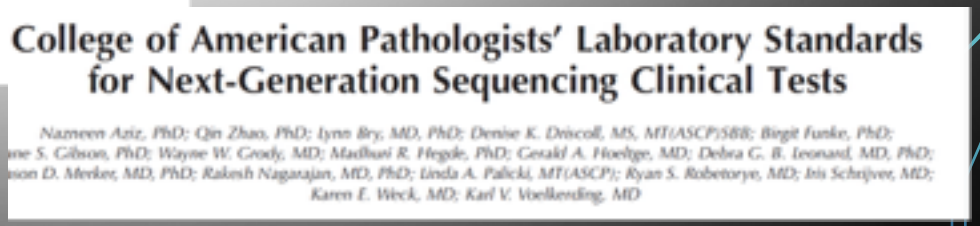

DOI 10.1186/s40246-015-0031-5

Human Genomics

REVIEW Open Access

Clinical application of next-generation sequencing for Mendelian diseases

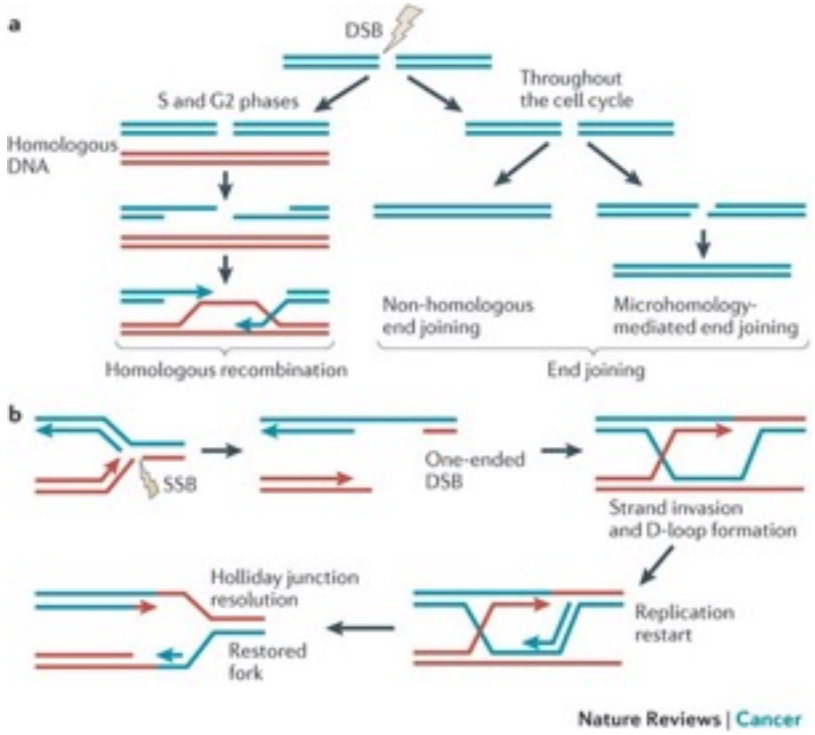
Saumya Shekhar Jamuar[1,2] and Ene-Choo Tan[2,3*]

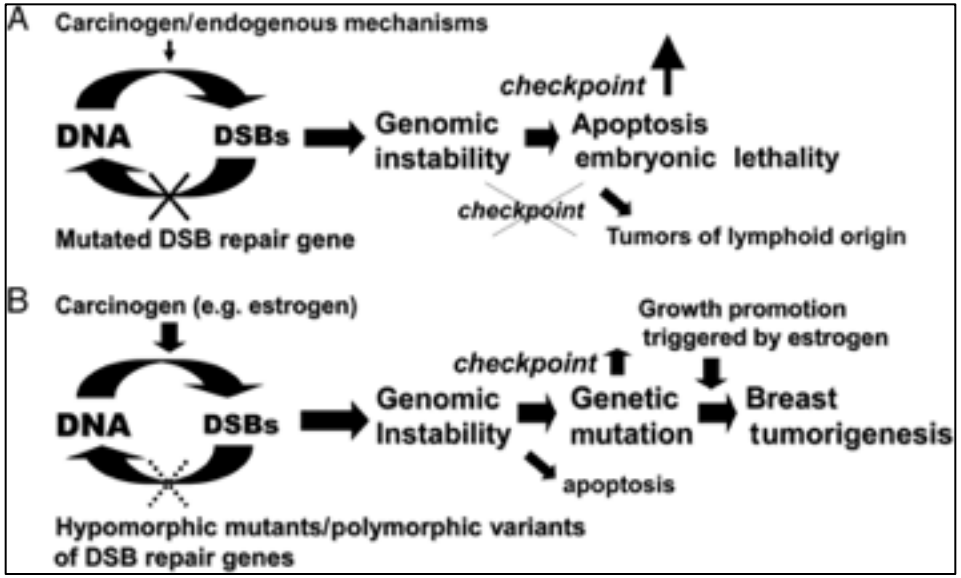

College of American Pathologists' Laboratory Standards for Next-Generation Sequencing Clinical Tests

Nazneen Aziz, PhD; Qin Zhao, PhD; Lynn Bry, MD, PhD; Denise K. Driscoll, MS, MT(ASCP)SBB; Birgit Funke, PhD; ne S. Gibson, PhD; Wayne W. Grody, MD; Madhuri R. Hegde, PhD; Gesaki A. Hoeltge, MD; Debra G. B. Leonard, MD, PhD; son D. Merker, MD, PhD; Rakesh Nagarajan, MD, PhD; Linda A. Palicki, MT(ASCP); Ryan S. Robetorye, MD; Iris Schrijver, MD; Karen E. Weck, MD; Karl V. Voelkerding, MD

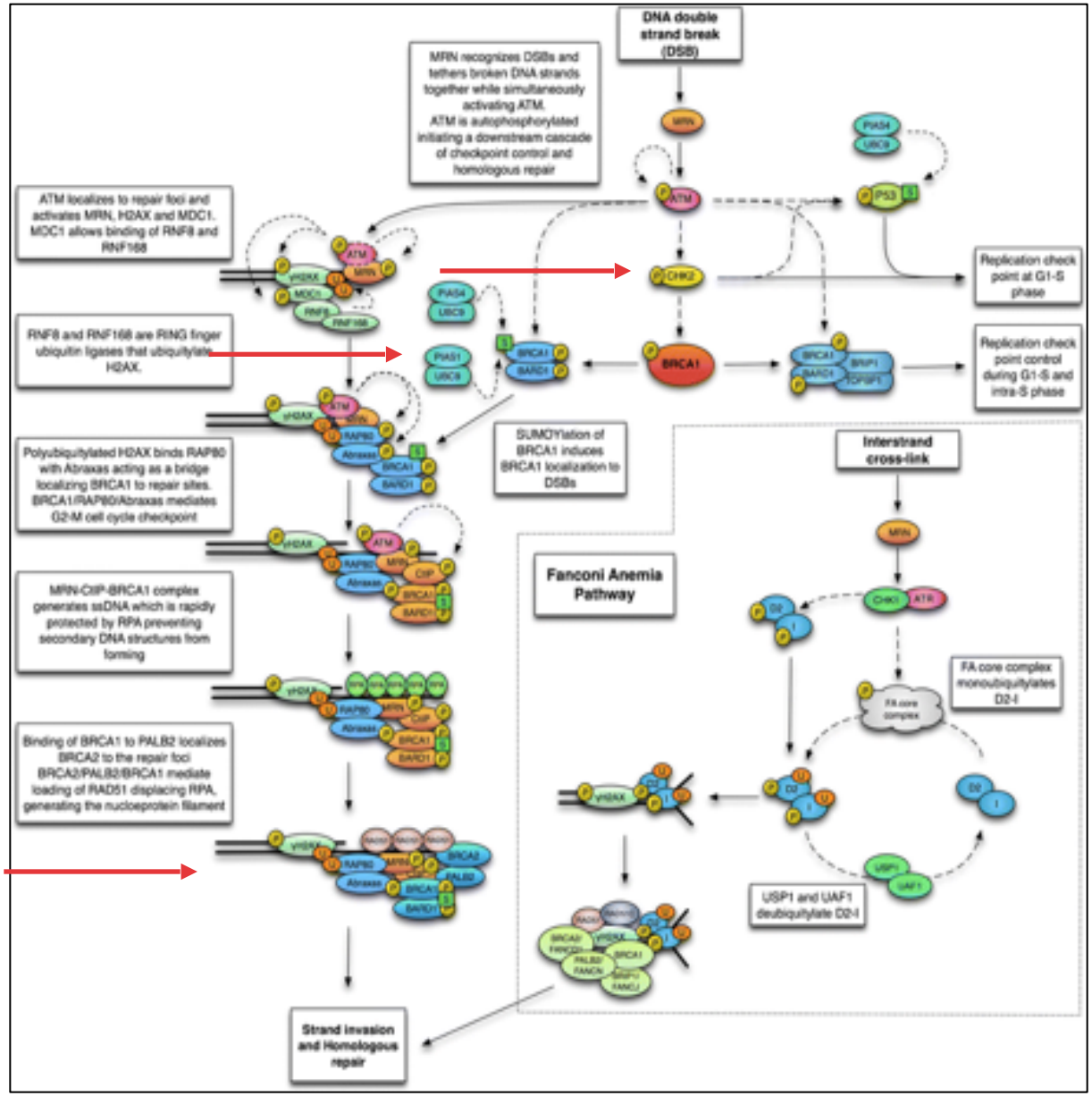
BRCA-1 protein and Double Strand Breaks (DSB)

By-products of the cell's own metabolism such as reactive oxygen species (ROS) can damage DNA bases and cause lesions that can block progression of replication.
The result is double-strand breaks (DSBs) in the chromosome.
DSBs can also be caused by **environmental exposure to irradiation, chemical agents, or ultraviolet light (UV)**

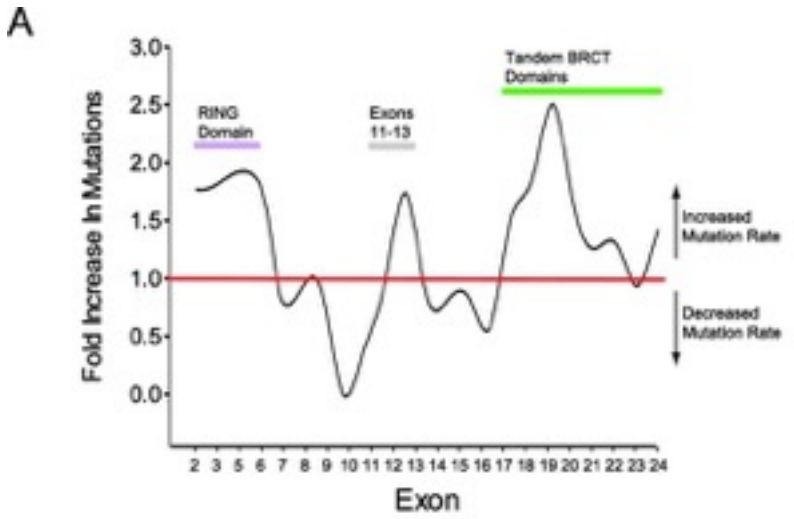


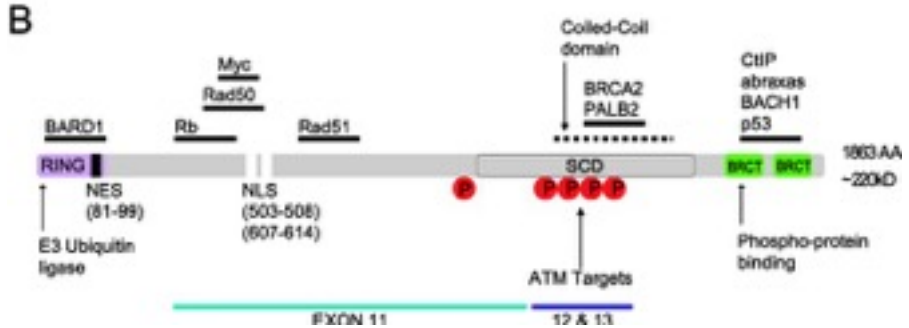


DSB and homologous recombination DNA repair pathway

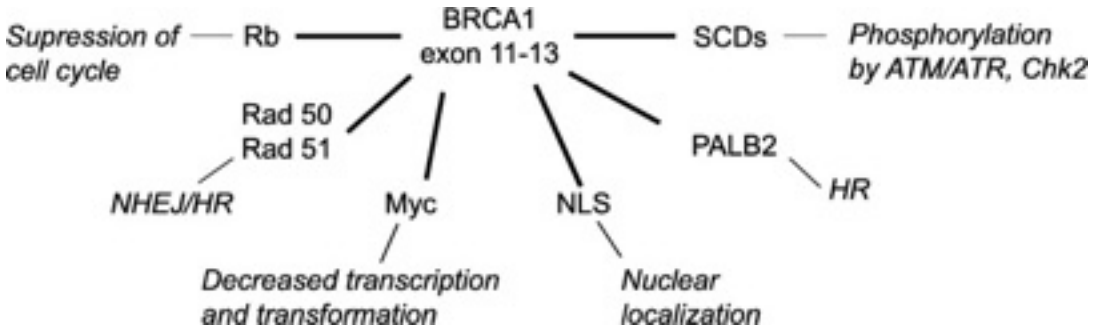


BRCA1 structure and function





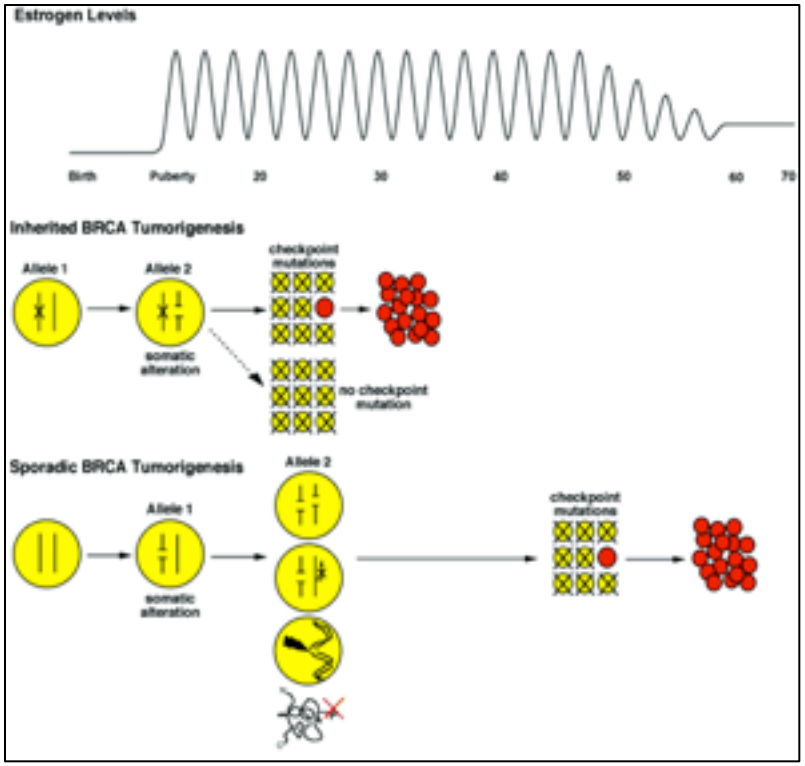
Clark et al.2012



Breast cancer

BRCA1/BRCA2 :

a particular way to be a tumor-suppressor gene



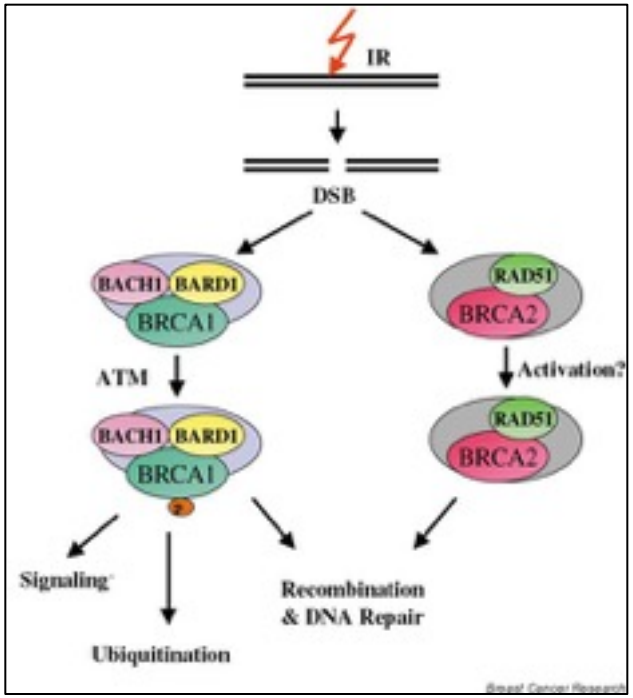
BRCA1/2 do not drive the sporadic breast tumourigenesis, which is characterized by chromosomal rearrangements (CIN)

CGH and gene expression analyses show a clusterization of the breast tumours with germline BRCA1 mutations (egs. TNBC)

Breast cancer

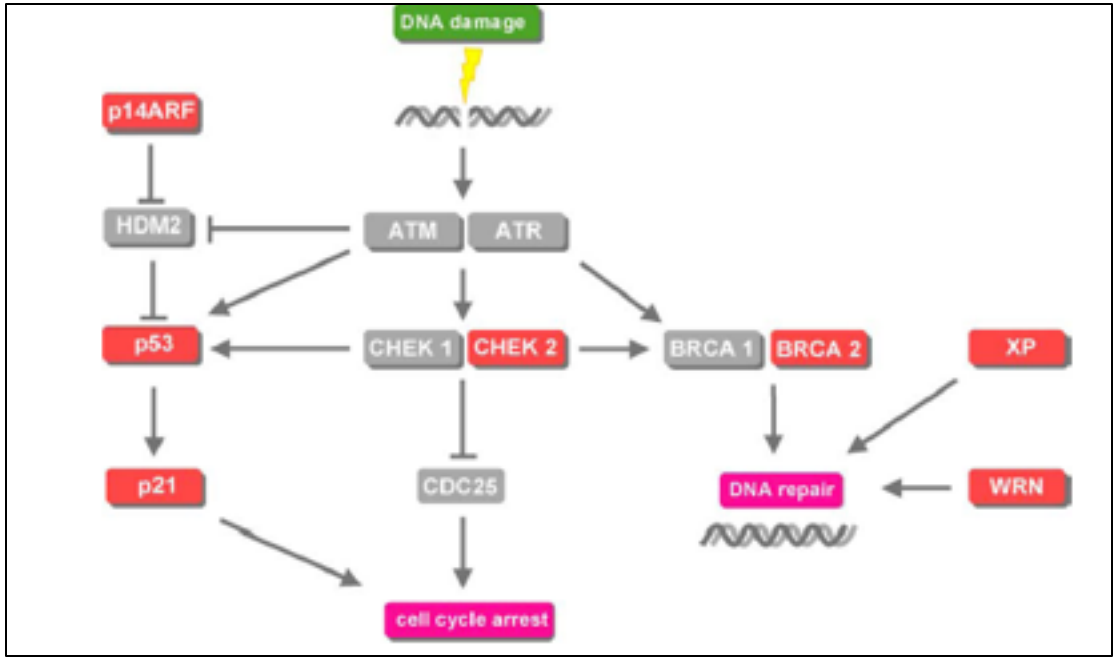
Low penetrant alleles

CHECK2 , ATM, TGFB1, CASP8



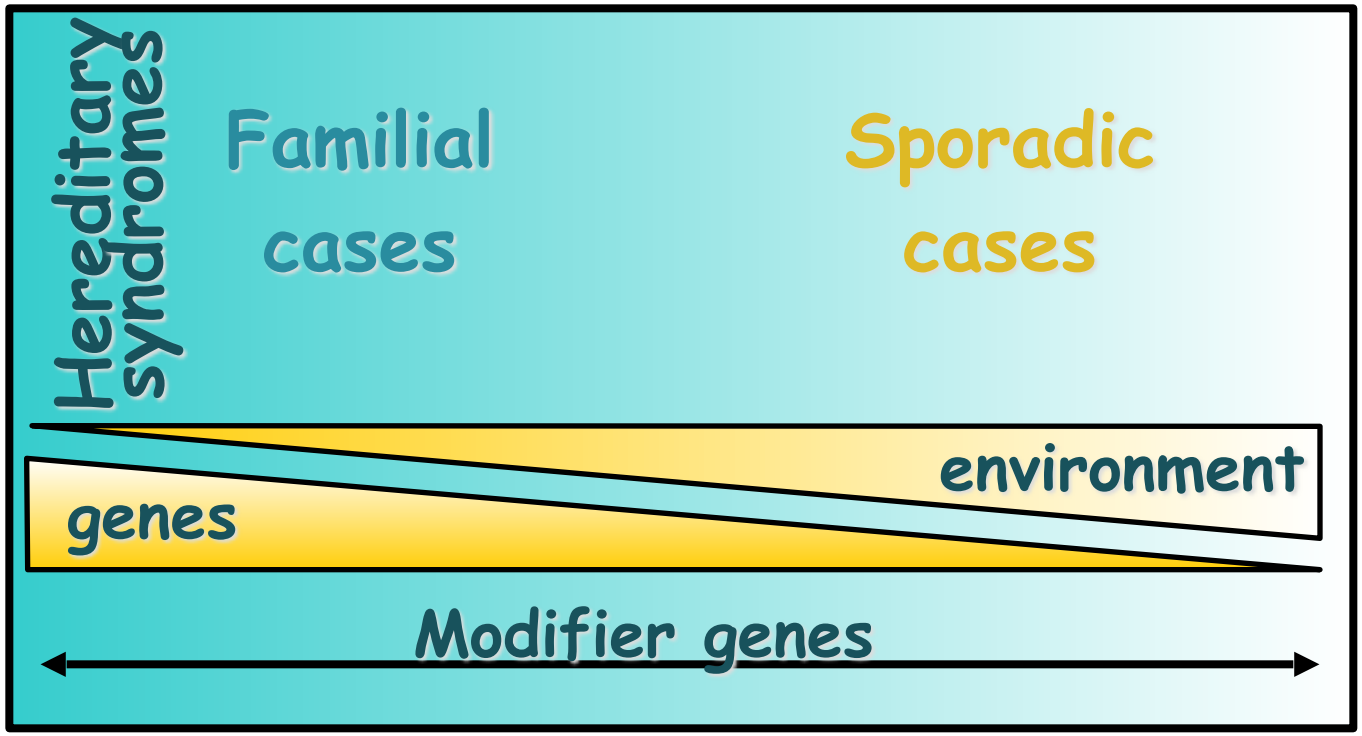
CHECK2 : 22q12.1; it is a G2 checkpoint kinase, activated by ultraviolet radiation through ATM phosphorylation

Variants can modulate BRCA 1 mutated phenotype



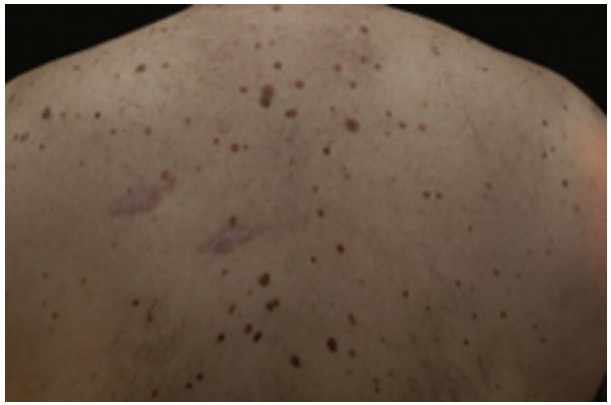
CHECK2 and ATM are on the BRCA1 pathway

Melanoma



Gene-environment interaction increases

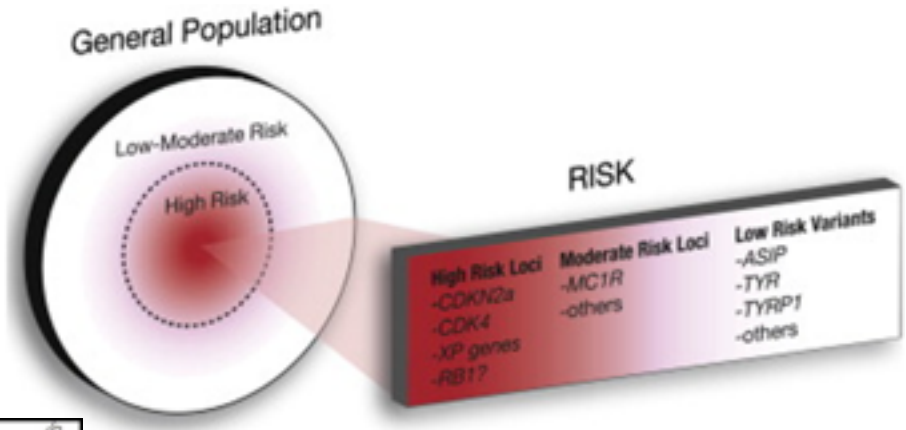
Melanoma

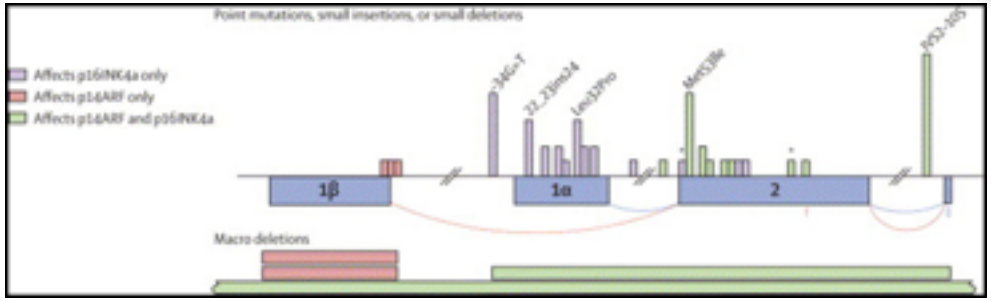


Melanoma are hereditary in 10% of cases: a) several melanomas in different generations, b) multiple primary melanomas in one subject, c) early onset of the disease

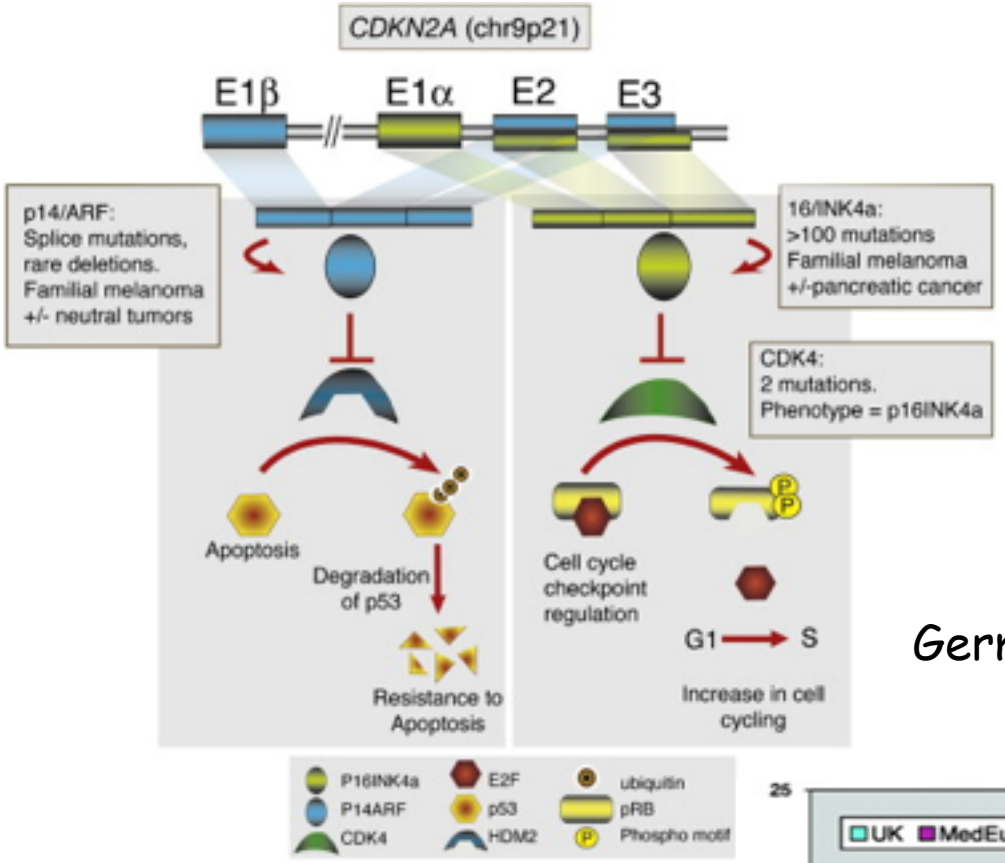
Autosomic dominant condition in linkage with loci on 9p21

Linkage analysis identified deleterious **CDKN2A** mutations (9p21) in association with hereditary melanoma in 1997





CDK2NA is composed of 4 exons and codes for 2 proteins : **p16/Ink4a** and **p14/Arf**

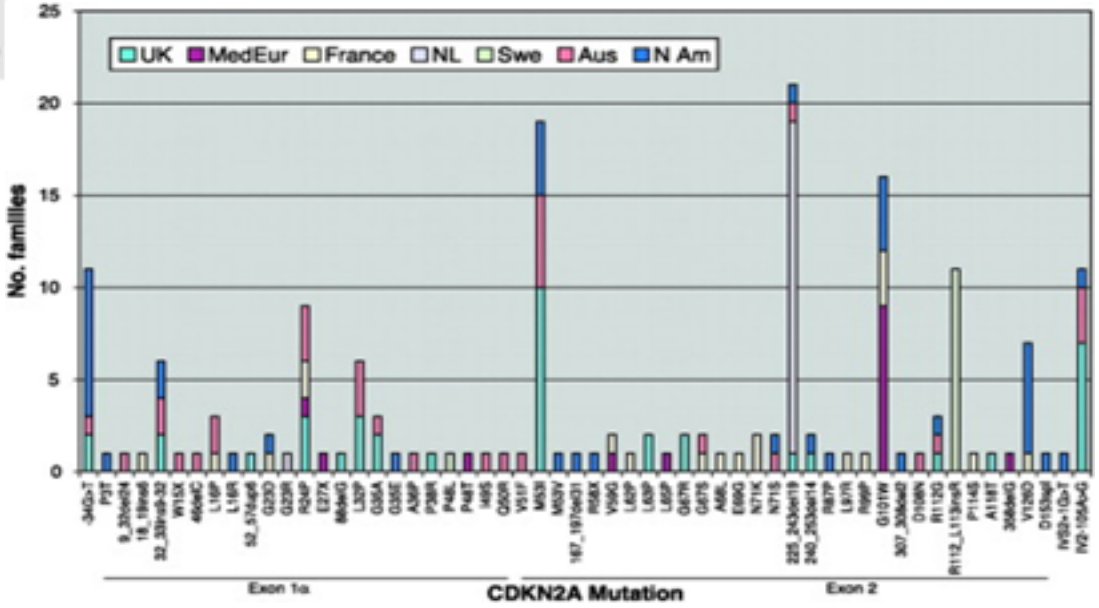


p16 loss induces the G1/S transition (by Rb1 phosphorylation)

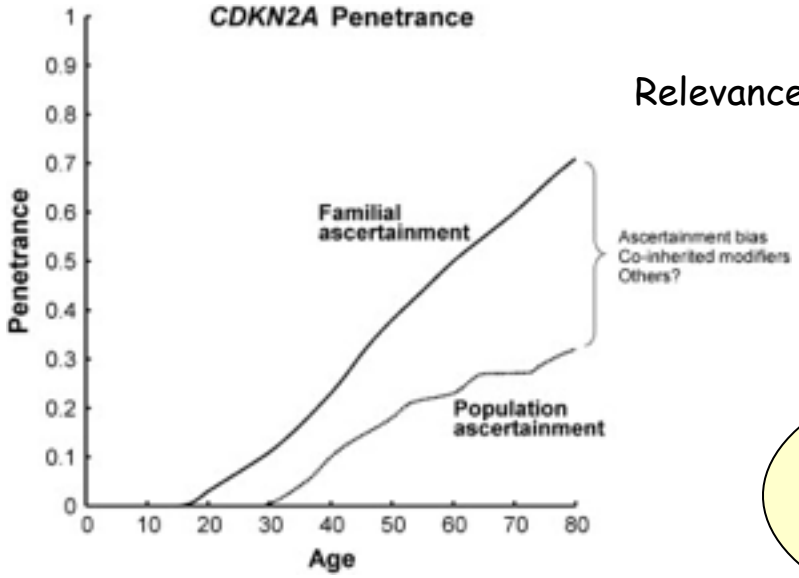
p14 loss makes p53 unstable

Germline mutations of these two genes affect 39% of the families.

CDKN2A germline mutations are frequently found in geographical region with low incidence of sporadic melanoma



CDKN2A germline mutations show a penetrance of 30% at 50 years and 67% at 80 years depending on the geographical region



Relevance of modifier factors (ex. UV exposition)

High pentrant alleles:

CDKN2A, ARF, CDK4

Low penetrant alleles:

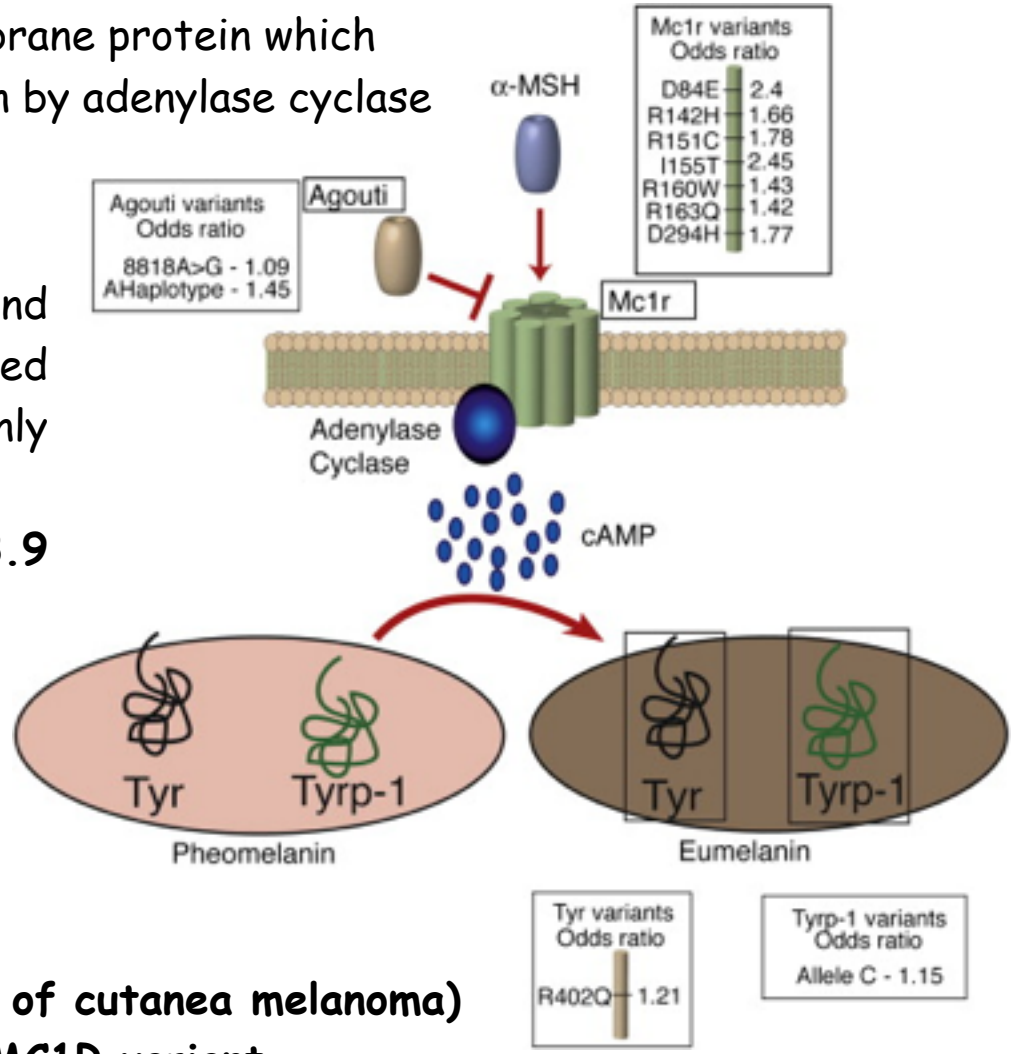
MC1R , vitamin D receptor

Phenotype is correlated with pigmentation, skin type, sun sensibility etc…

More than 120 genes are involved in pigmentation and skin colour...

MC1R codifies for a transmembrane protein which activates the eumelanin production by adenylase cyclase

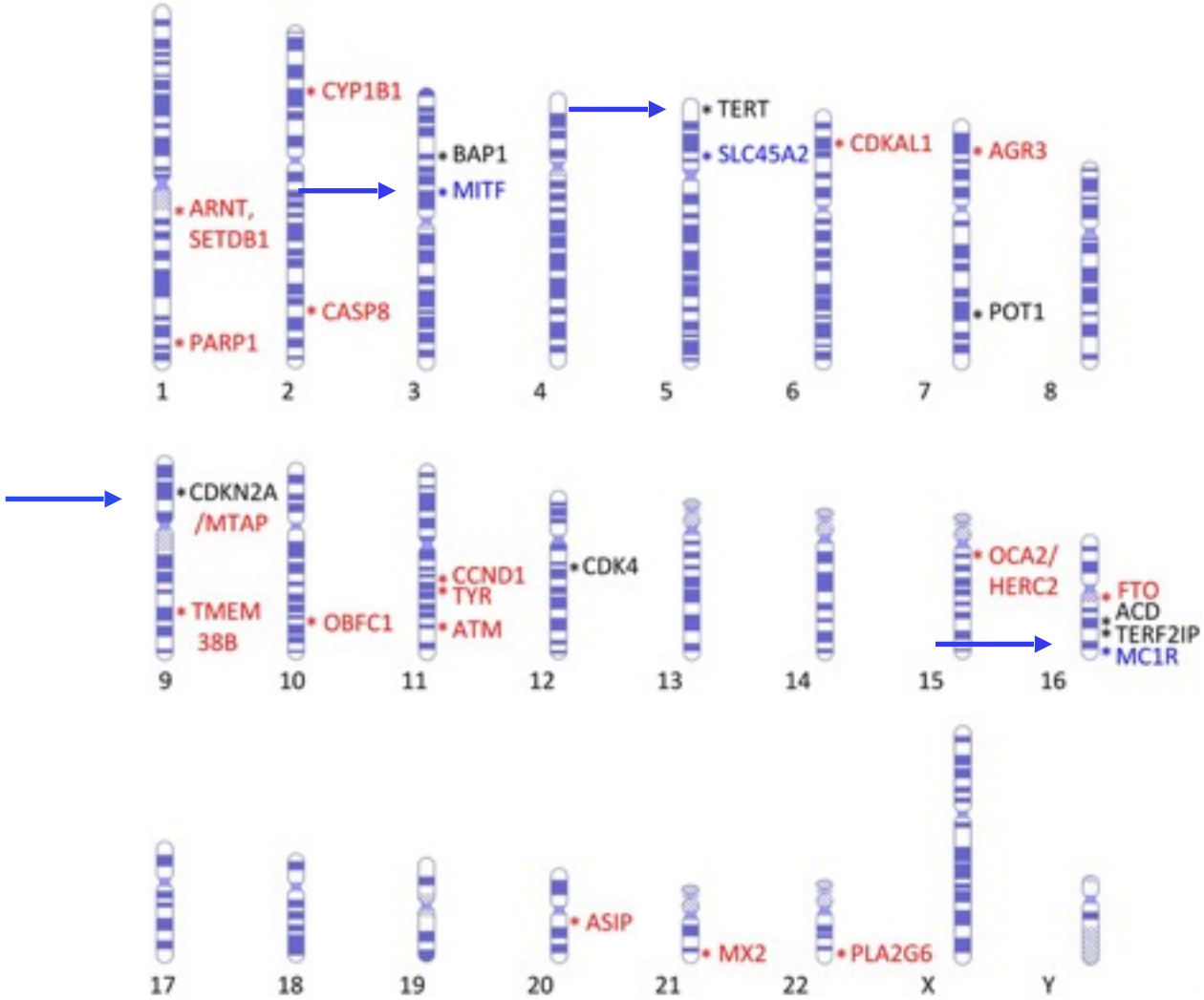
- These genetic variants are found in 80% of individuals with red hair and clear skin, but in only 20% of those with black hair
- They are associated with a **3.9** higher risk of melanoma



MC1R plays a role as a phenotype modifier in association with CDKN2A

Somatic BRAF mutations (50% of cutanea melanoma) are associated with MC1R variant

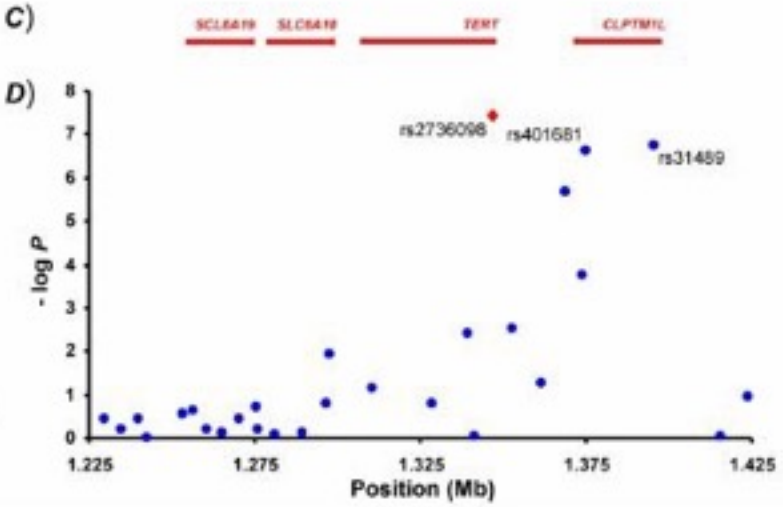
High, medium and low penetrance genes and their chromosome band locations.



Jazlyn Read et al. J Med Genet 2016;53:1-14

Finally, a variant that affects risk of many types of cancer !

- GWA study on basal cell carcinoma (BCC) identified several regions that associate with increased risk of skin cancer (Stacey et al 2008)
- One on chr5p near two known "cancer genes"
  - *CLPTM1L* (cisplatin resistance related protein) gene
  - **h*TERT*** (human telomerase reverse transcriptase) gene

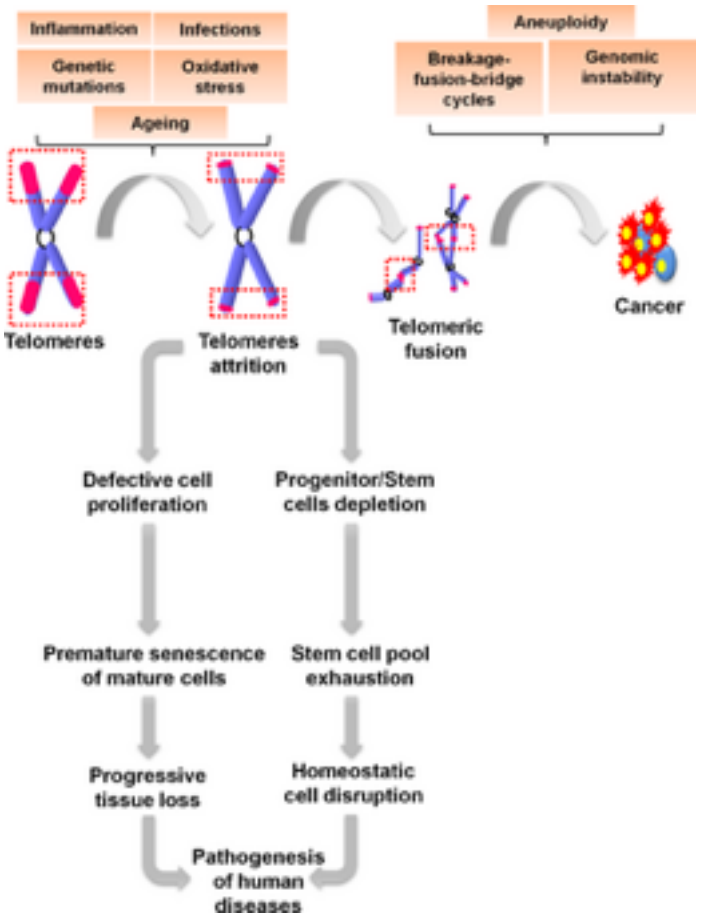


deCODE genetics

rs401681 (C) associates with risk of cancer at 5 sites

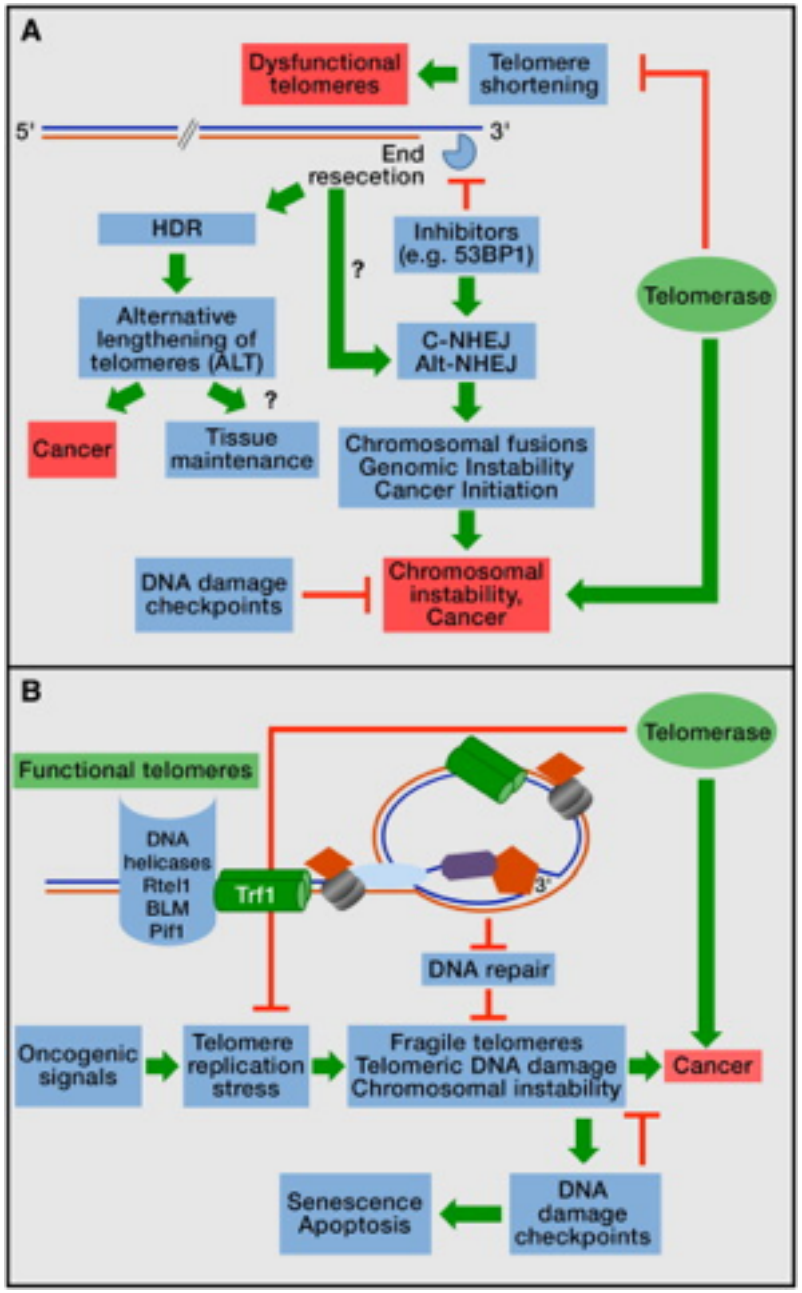
Study population	Number		Frequency		OR	95% CI	*P* value
	Cases	Controls	Cases	Controls			
Basal cell carcinoma							
Iceland all	2,040	28,890	0.604	0.545	1.27	1.19-1.36	9.5×10^{-12}
Eastern Europe	525	515	0.616	0.575	1.16	0.97-1.39	0.098
All combined	**2,565**	**515**	**0.610**	**0.560**	**1.25**	**1.18-1.34**	$\mathbf{3.7\times10^{-12}}$
Lung cancer							
Iceland all	1,449	28,890	0.575	0.545	1.13	1.04-1.23	3.6×10^{-3}
The Netherlands	529	1,832	0.610	0.570	1.18	1.02-1.35	0.021
Spain	367	1,427	0.582	0.538	1.19	1.01-1.41	0.034
IARC	1,920	2,517	0.617	0.586	1.16	1.06-1.27	8×10^{-4}
All combined	**4,265**	**34,666**	**0.596**	**0.560**	**1.15**	**1.10-1.22**	$\mathbf{7.2\times10^{-8}}$
Bladder cancer							
Iceland all	780	28,890	0.583	0.545	1.16	1.05-1.29	4.5×10^{-3}
The Netherlands	1,277	1,832	0.584	0.570	1.06	0.96-1.17	0.27
UK	707	506	0.564	0.514	1.23	1.04-1.44	0.014
Italy-Torino	329	379	0.550	0.545	1.02	0.84-1.24	0.84
Italy-Brescia	122	156	0.574	0.564	1.04	0.74-1.46	0.82
Belgium	199	378	0.603	0.554	1.22	0.95-1.56	0.11
Eastern Europe	214	515	0.619	0.575	1.20	0.96-1.51	0.12
Sweden	346	905	0.545	0.521	1.10	0.92-1.31	0.30
Spain	173	1,427	0.546	0.538	1.03	0.83-1.29	0.78
All combined	**4,147**	**34,988**	**0.578**	**0.535**	**1.12**	**1.06-1.18**	$\mathbf{5.7\times10^{-5}}$
Prostate cancer							
Iceland all	2,276	28,890	0.569	0.545	1.10	1.03-1.17	3.75×10^{-3}
The Netherlands	994	1,832	0.576	0.570	1.02	0.92-1.14	0.67
Chicago, US	635	693	0.581	0.568	1.06	0.90-1.23	0.49
Spain	459	1,427	0.559	0.538	1.09	0.94-1.26	0.27
CGEMS	5,109	5,059	0.558	0.543	1.06	1.00-1.11	0.036
All combined	**9,473**	**37,901**	**0.569**	**0.553**	**1.07**	**1.03-1.11**	$\mathbf{3.6\times10^{-4}}$
Cervical cancer							
Iceland all	369	28,890	0.611	0.545	1.31	1.13-1.51	2.6×10^{-4}

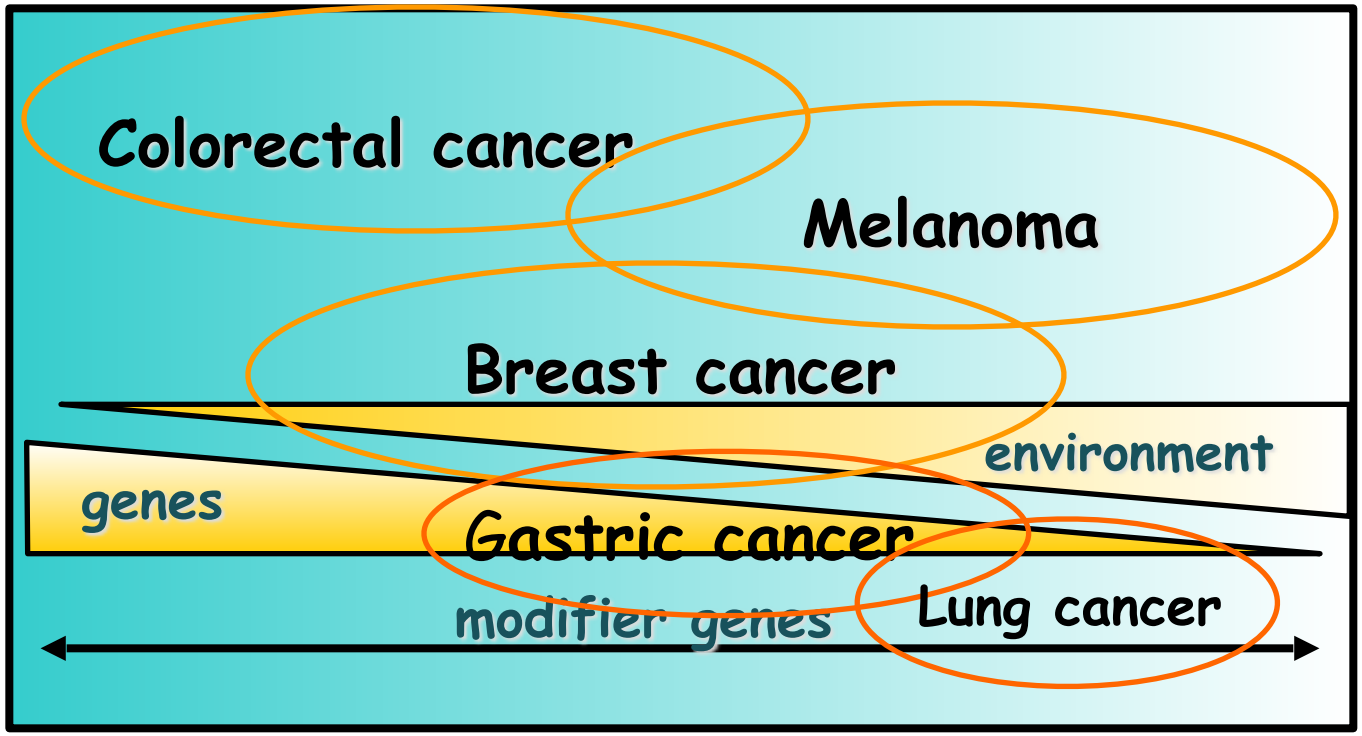
Defects in telomere- and telomerase-associated proteins lead to progressive telomere shortening

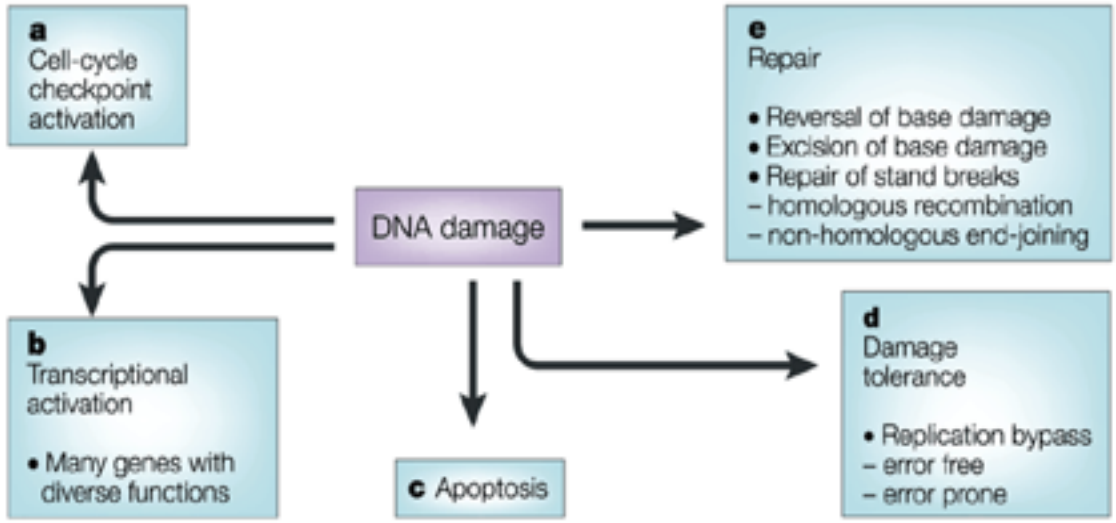


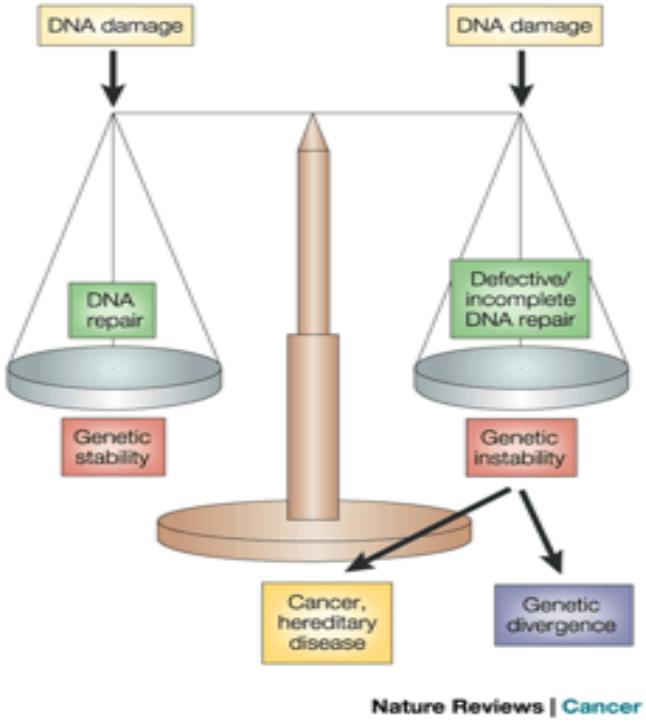
1. Telomere attrition coupled with deficiency in telomerase activity culminates in **replicative senescence in adult stem cells**, leading to a **depletion** in the stem cell reserve. Collectively, these eventually manifest as hematological (i.e. anaplastic anemia, pancytopenia, bone marrow failure) or non hematological (i.e. skin abnormalities, pulmonary diseases, liver diseases) clinical features.

2. In **adult cells** short dysfunctional telomeres inevitably result in telomeric fusions, leading to **genome instability**, the cornerstone for carcinogenesis.


A
Dysfunctional telomeres
Telomere shortening
5'
3'
End resecetion
HDR
Inhibitors (e.g. 53BP1)
?
Telomerase
Alternative lengthening of telomeres (ALT)
C-NHEJ Alt-NHEJ
?
Cancer
Tissue maintenance
Chromosomal fusions Genomic Instability Cancer Initiation
DNA damage checkpoints
Chromosomal instability, Cancer
B
Telomerase
Functional telomeres
DNA helicases Rtel1 BLM Pif1
Trf1
3'
DNA repair
Oncogenic signals
Telomere replication stress
Fragile telomeres Telomeric DNA damage Chromosomal instability
Cancer
Senescence Apoptosis
DNA damage checkpoints


Colorectal cancer
Melanoma
Breast cancer
environment
genes
Gastric cancer
modifier genes
Lung cancer





Mutational load at any given time in both germ line and somatic cells is the outcome of a **dynamic equilibrium** between the **extent of DNA damage**, the efficiency of **DNA repair**

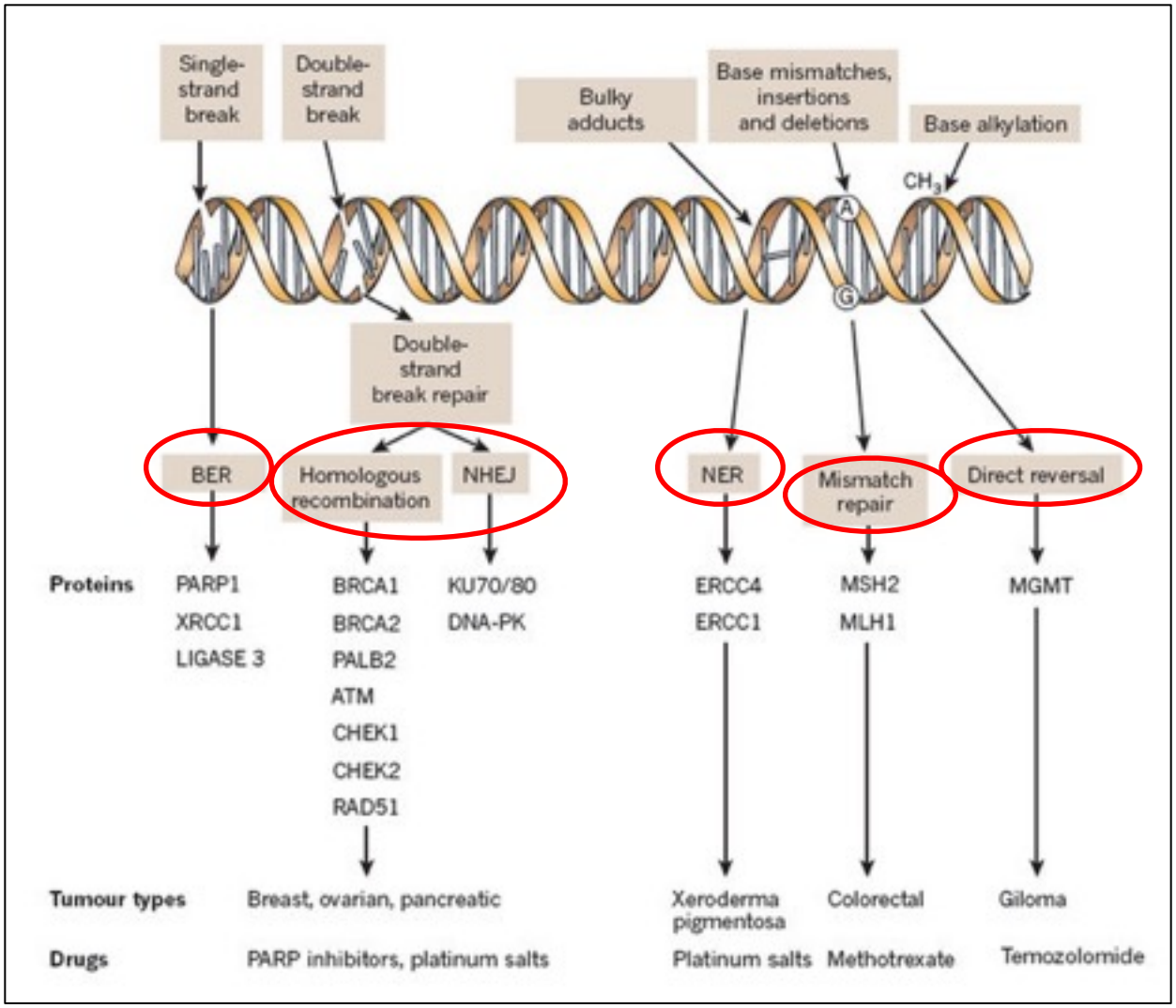
DNA alterations

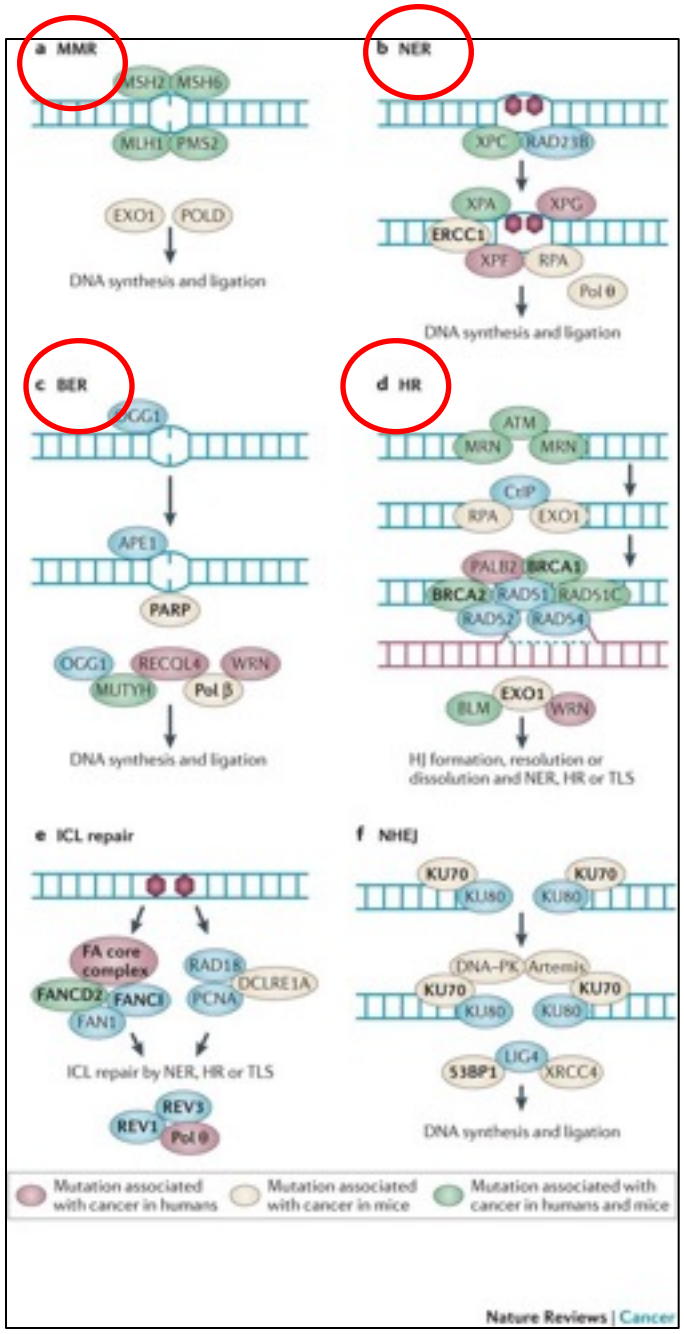
- **Depurination** : loss of adenine/guanine residues for spontaneous fission of bonds between base and sugar; very common about 5000 purine bases are lost each day from a cell
- **Deamination** : from cytosine to uracil; daily 100 bases per human cell
- **Reactive oxygen species** (ROS) : ROS are caused by ionization rays, secondary products of cell respiration
- **Replication errors/recombination**: damaged sequence is excised and the gap is filled

130 gene are involved in this process
(www.cgal.icnet.uk/DNA_repair_Genes.htlm)

5 different evolutionary conserved systems of repair are engaged

DNA repair pathways and cancers





DNA REPAIR SYSTEMS

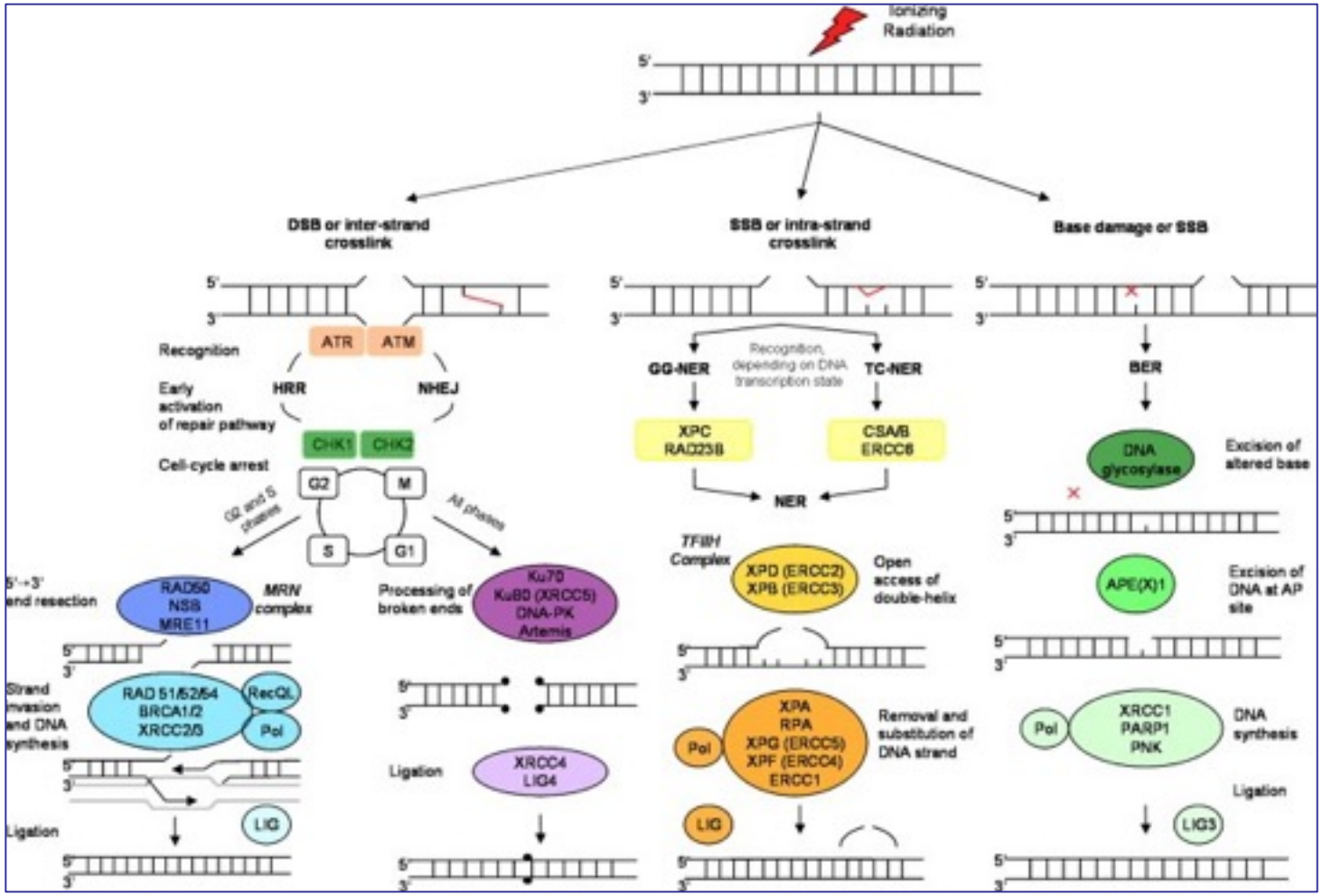
- The nucleotide excision repair or **NER** pathways repairs **bulky helix distorting adducts** such as thymine dimers induced by UV

- Base excision repair or **BER** removes DNA bases that are damaged by **oxidation**

- DNA double strand break (DSB) repair by homologous recombination **HR** is active in late S or G2 phases of the cell cycle

  - Interstand crosslinks (ICL) repair is a combination of different pathways, including NER, HR and translation synthesis polymerases

  - Non homologous end-joining (NHEJ) is active throughout the cell-cycle and is the only DSB pathway that is available in G1 phase when there are no templates for HR



A lot of DNA repair proteins are common with translational or recombination pathways

NER pathways

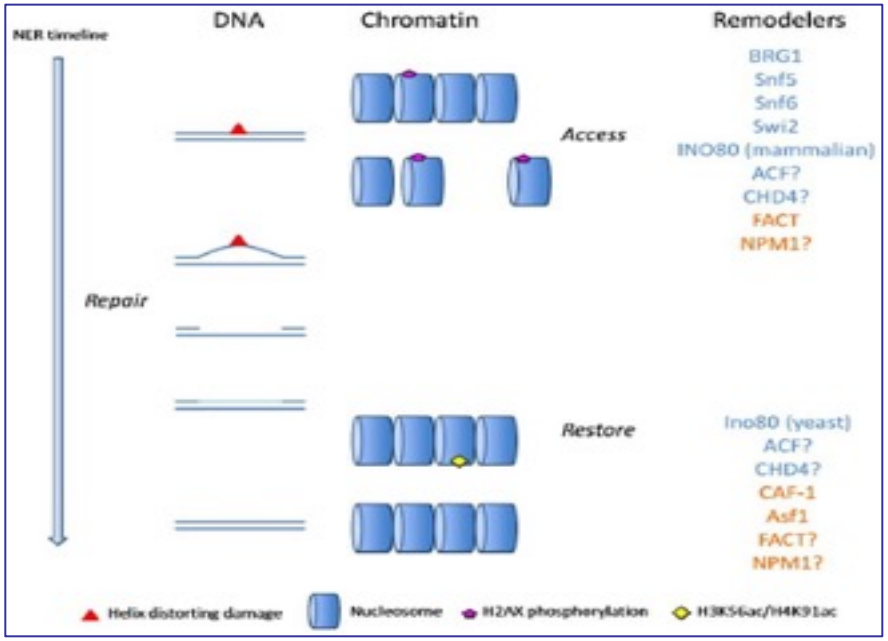
- **Nucleotide excision repair (NER) eliminates various structurally unrelated DNA lesions by a multiwise 'cut and patch'-type reaction.**

- NER detects and removes DNA lesions within the **chromatin**

- Lesions not only has to activate the NER pathway but also a preliminary **chromatin remodeling** to access DNA

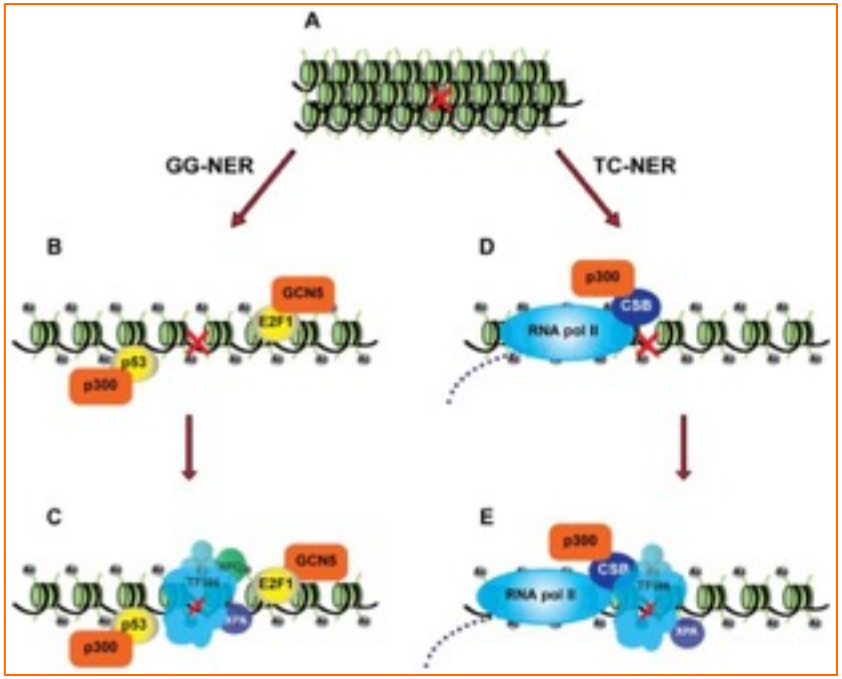
- Cooperating mechanisms enhance the efficiency of NER by **altering chromatin structure**.

- Many of the players were identified as **regulators of transcription** (ATP-dependent chromatin remodelers, histone modifying enzymes and several transcription factors)



The **global genome NER (GG-NER)** pathway prevents mutagenesis by detecting the genome for helix-distorting lesions

The **transcription-coupled NER (TC-NER)** removes transcription-blocking lesions to allow gene expression



- ***The p53 and E2F1 transcription factors** are well known to <u>regulate gene expression in response to DNA damage</u> by contributing to modifying chromatin structure in response to DNA damage to promote global NER*

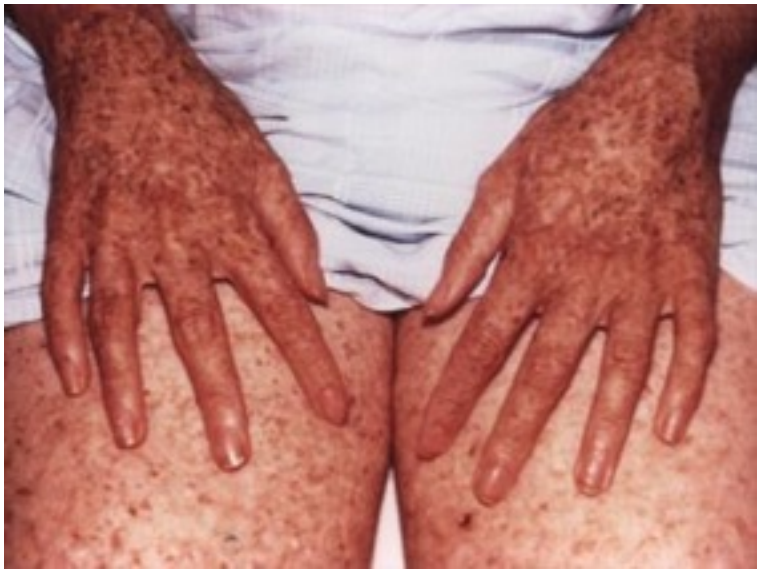
- ***After completion of the repair, the chromatin must be returned to its previous undamaged state** (post-translational modifications and insertion of histone variants and displacement)*

Defects in GG-NER result in cancer predisposition

Defects in TC-NER cause a variety of diseases
(i.e. from ultraviolet radiation-sensitive syndrome to severe premature ageing conditions)

*Humans with a hereditary defect in NER suffer from **xeroderma pigmentosum***

*They have a marked **predisposition to skin cancer** caused by sunlight exposure.*



The balance between GG-NER and TCR is crucial for protection from cancer as well as from premature ageing

*In **TCR deficiency** this balance is disturbed, and cells with a low DNA damage load die from transcriptional stress, resulting in accelerated ageing and strong protection from cancer.*

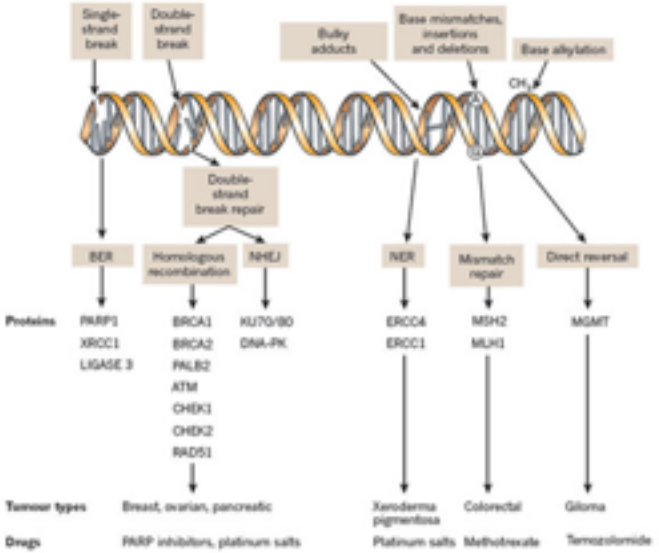
*In **GG-NER deficiency**, the still functional TCR promotes cell survival and delays ageing at the expense of accumulating DNA damage in non transcribed sequences, including the non transcribed strand of actively expressed genes*

Tumors with specific DNA repair defects can be **completely dependent on these DNA repair pathways**

This can be used for therapies
(i.e. homologous recombination deficient tumors can be targeted by DNA double-strand-break-inducing agents)

This dependence can be exploited therapeutically to induce **synthetic lethality** in tumors

Bouwman and Jankers, 2012



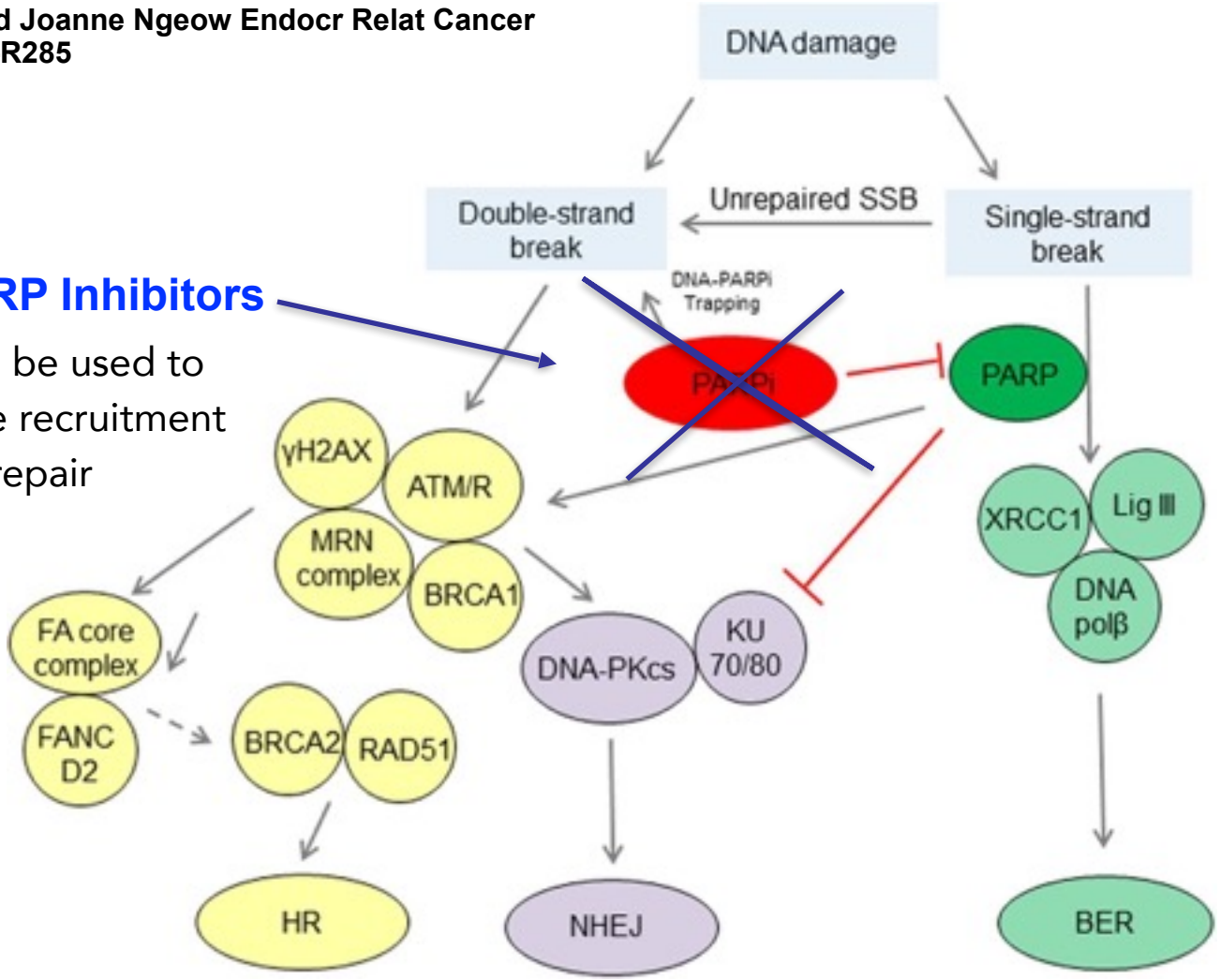
Tumors cells derived from patients **carrying germline defects** in key regulator genes of DNA repair pathways could be a useful model to understand responsiveness to synthetic lethality therapies
(eg. **BRAC1/BRCA2 with PARP inhibitors**)

PARP1 binds to DNA single-strand break and catalyzes poly(ADP)ribosylation of itself and acceptor proteins, facilitating the recruitment of DNA repair proteins

Diana Lim, and Joanne Ngeow Endocr Relat Cancer 2016;23:R267-R285

PARP Inhibitors

They can be used to block the recruitment of DNA repair proteins



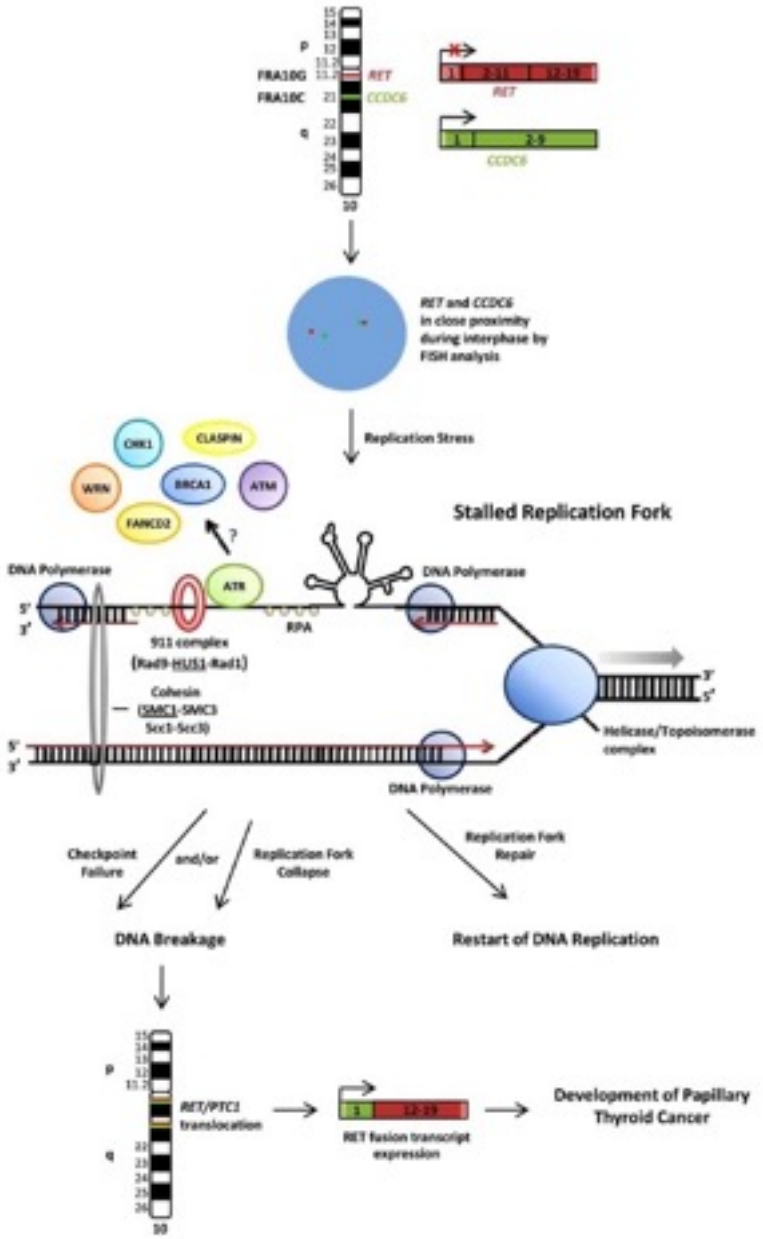
FRAGILE SITES AND CANCER

- Fragile sites are specific genomic regions, *mendelian inherited*, exhibiting gaps or breaks on metaphase chromosomes f*ollowing conditions of partial replication stress.*

- Fragile sites often *coincide with genes that are frequently rearranged or deleted in human cancers* (examples : formation of *RET/PTC* rearrangements, and deletions within the *FHIT* gene)

- It is hypothesized that under replication stress, *stable secondary structures form at fragile sites and stall replication fork progress, ultimately resulting in DNA breaks.*

- The *ATR DNA* damage checkpoint pathway plays a critical role in maintaining stability at fragile sites.



- Genomic instability occurs preferentially at fragile sites (FS), *evolutionarily conserved* and late replicating regions with AT-rich sequences.

- Identifying a cancer-associated FS gene (CACG) remains a challenge and little is known about the function of CACGs at most FS loci.

- Recent studies of *FATS* (for Fragile-site Associated Tumor Suppressor), reveal an active role of these genes in regulating *DNA damage checkpoints* and suppressing tumorigenesis.

Human CFS	Location	Frequency	Associated genes	CACG
FRA2G	2q31	modest	*IGRP*, *RDHL*, *LRP2* and others	not validated
FRA2H	2q32	modest	non-coding RNA gene	not validated
FRA3B	3p14.2	high	*FHIT*	*FHIT*
FRA4F	4q22	modest	*GRID2*	not validated
FRA6E	6q26	modest	*PARK2*, *PLG*, *LPA* and others	*PARK2*
FRA6F	6q21	Modest	*REV3L*, *DIF13*, *FKHRL* and others	not validated
FRA7B	7p22	low	*THSD7A*, *SDK1*, *MAD1L1*	not validated
FRA7G	7q31.2	modest	*MET*, *TESTIN*, *CAV*, and others	*MET*, *TESTIN*
FRA7I	7q36	modest	*PIP*	not validated
FRA7K	7q31	modest	*IMMP2L*	not validated
FRA8C	8q24	modest	*MYC*	*MYC*
FRA9E	9q32	low	*PAPPA* and others	*PAPPA*
FRA10F	10q26	low	*FATS* and others	*FATS*
FRA10G	10q11	low	*RET*, *NCOA4*	*RET*
FRA16D	16q23.2	high	*WWOX*/*FOR*	*WWOX*
FRAXB	Xp22.3	modest	*STS*, *GS1*	not validated
FRAXC	Xq22.1	modest	*DMD*, *IL1RAPL1*	*DMD*