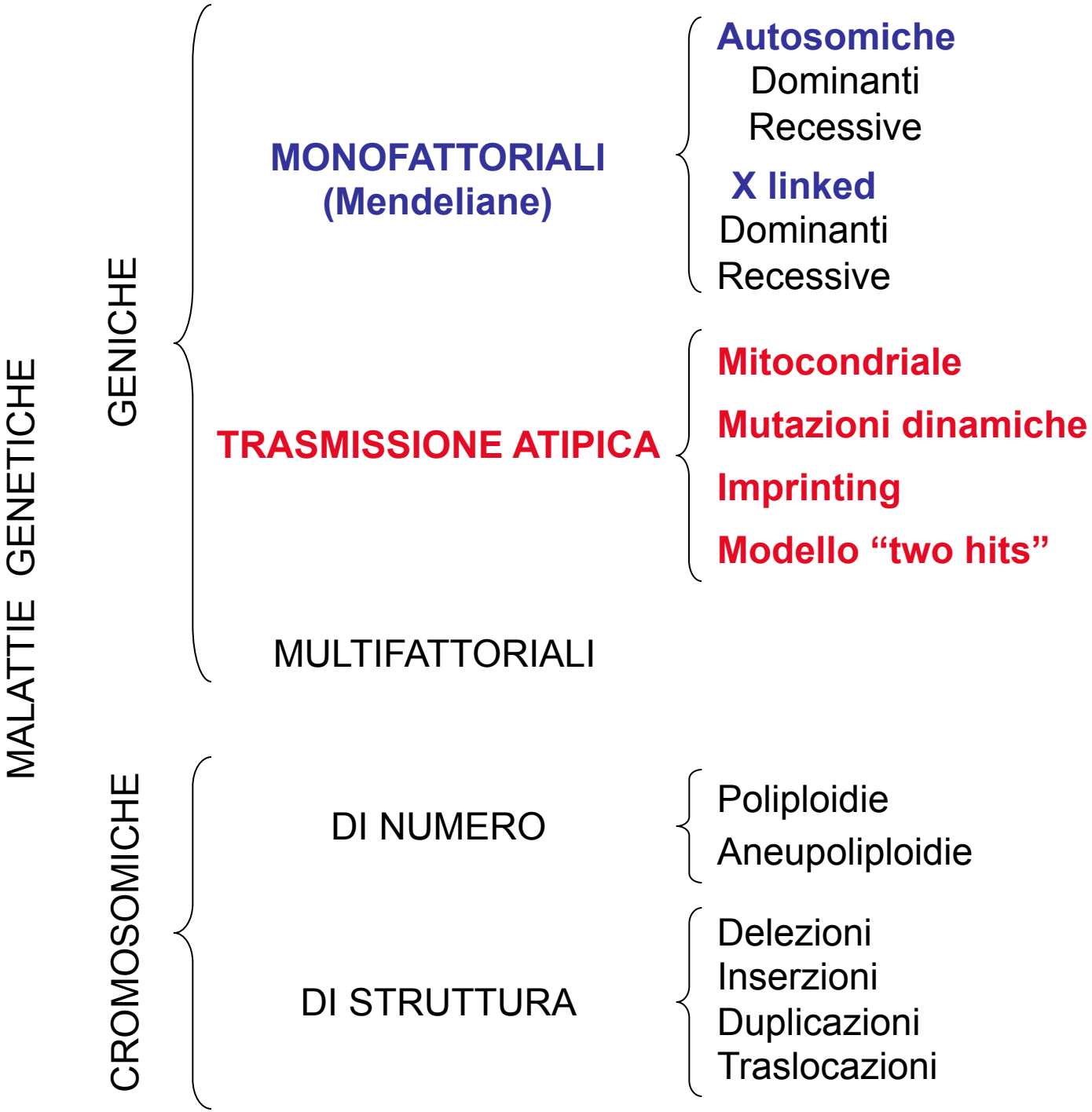

MALATTIE GENETICHE
GENICHE
MONOFATTORIALI
(Mendeliane)
Autosomiche
Dominanti
Recessive
X linked
Dominanti
Recessive
TRASMISSIONE ATIPICA
Mitocondriale
Mutazioni dinamiche
Imprinting
Modello "two hits"
MULTIFATTORIALI
CROMOSOMICHE
DI NUMERO
Poliploidie
Aneupoliploidie
DI STRUTTURA
Delezioni
Inserzioni
Duplicazioni
Traslocazioni

Organizzazione del Genoma Umano

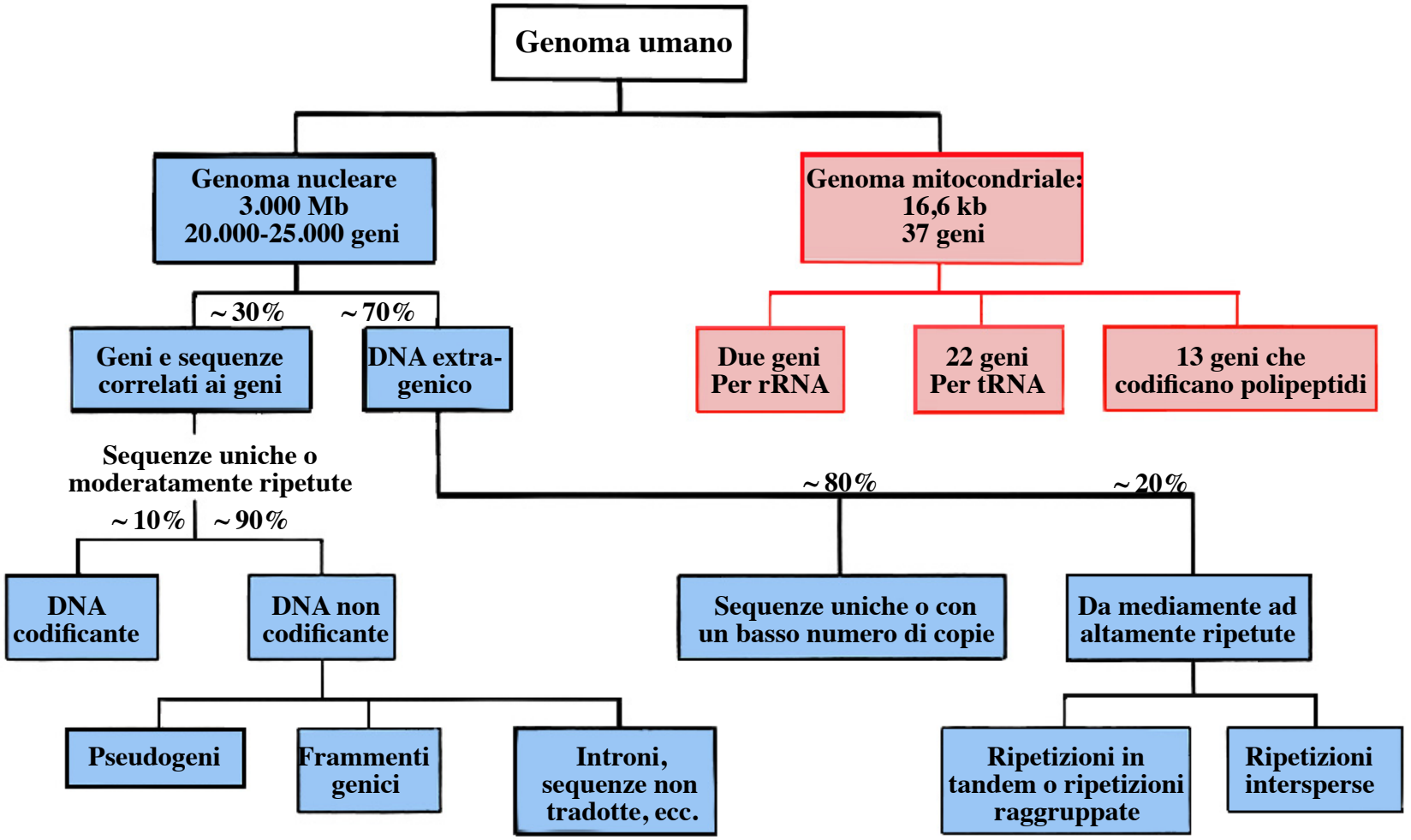
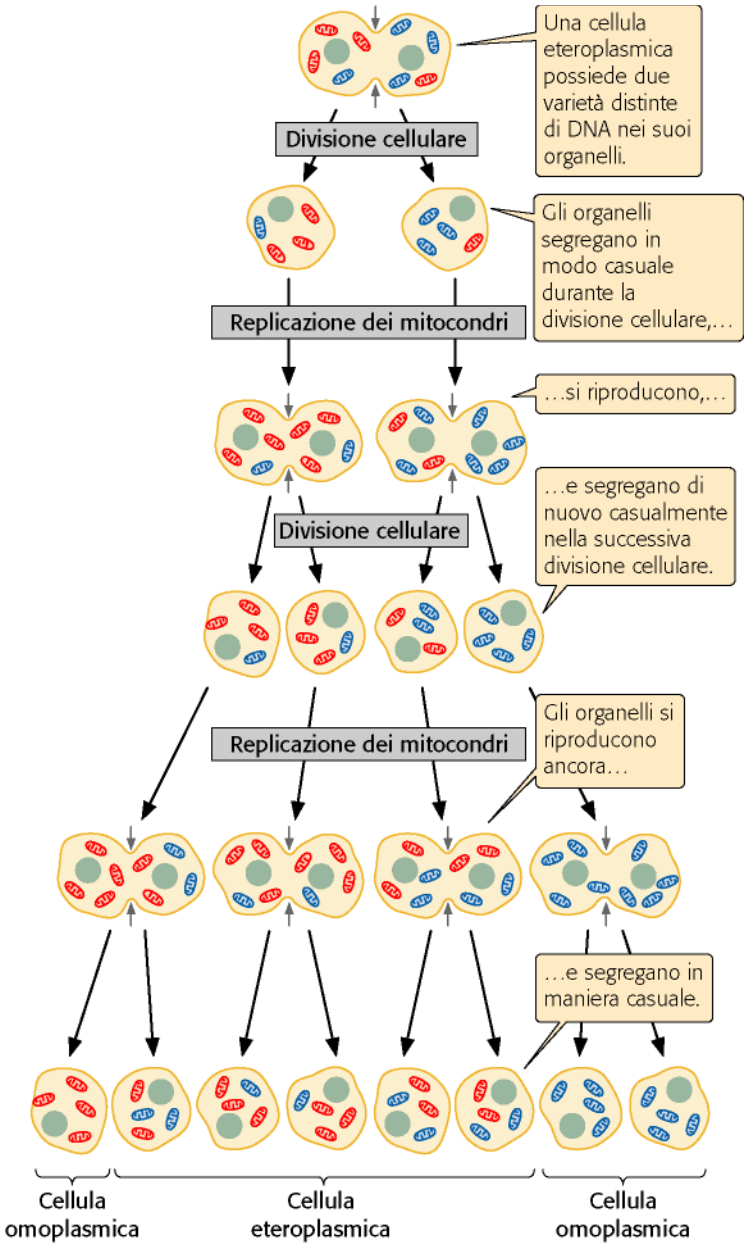


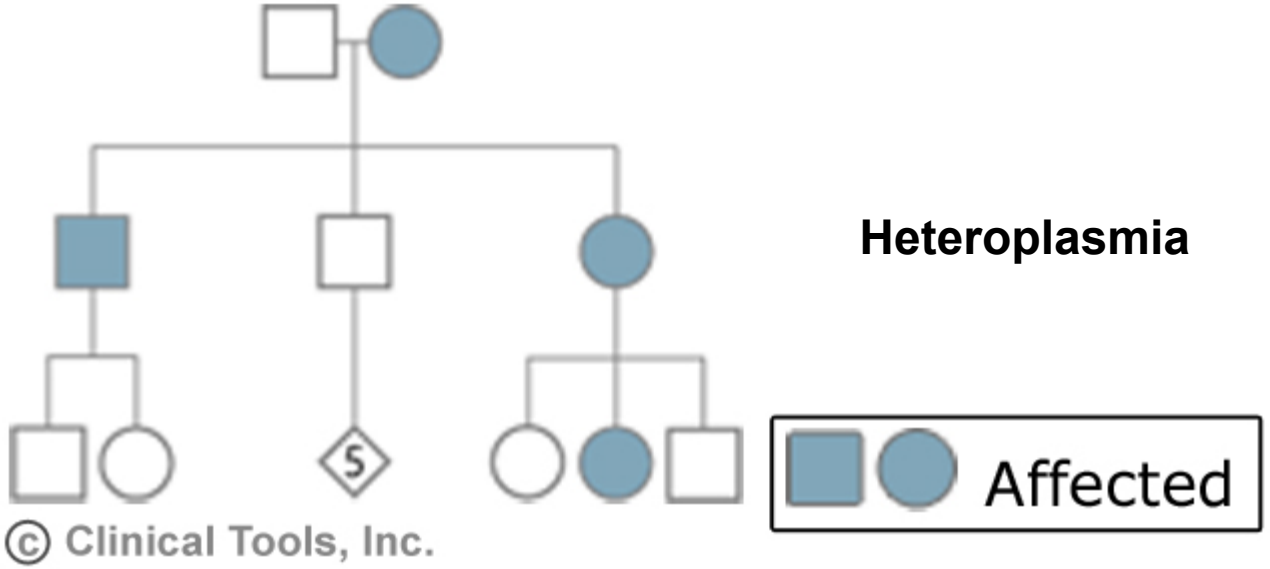
Tabella 7.1: Il genoma nucleare e il genoma mitocondriale nell'uomo

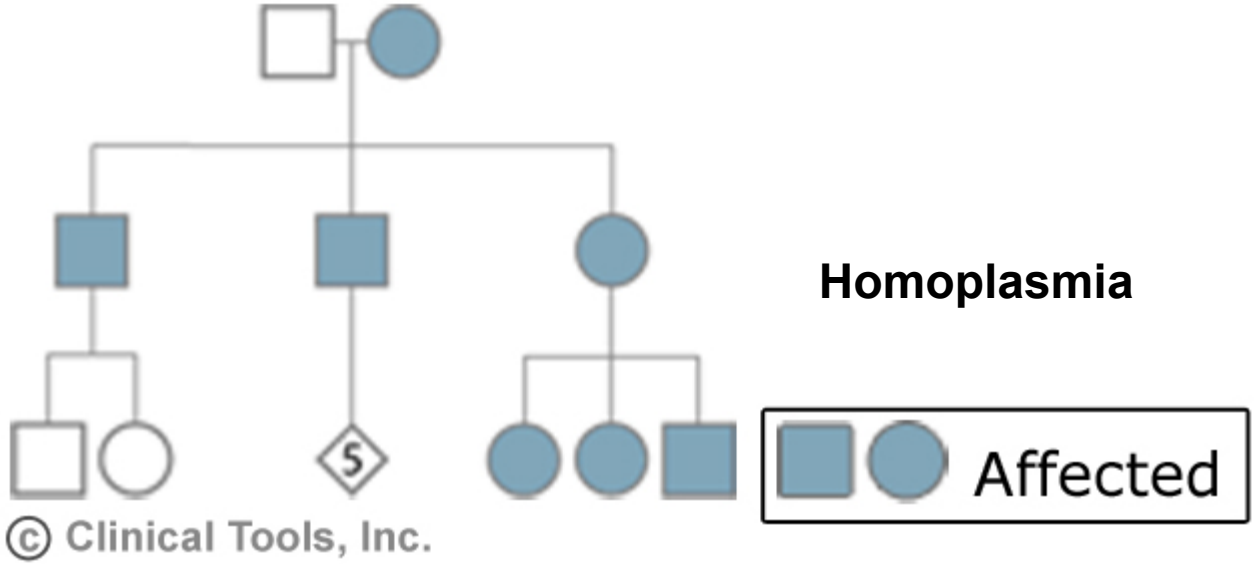
	Genoma nucleare	Genoma mitocondriale
Dimensione	3.300 Mb	16,6 kb
N° di molecole di DNA differenti	23 o 24 (nelle cellule XX o XY rispettivamente), tutte lineari	Una molecola di DNA circolare
N° totale di molecole di DNA per cellula	23 nelle cellule aploidi; 46 nelle cellule diploidi	Numerose migliaia
Proteine associate	Diverse classi di istoni e proteine non istoniche	Per lo più senza proteine
N° di geni	~ **20.000-25.000**	37
Densità genica	~ **1/100 kb**	1/0,45 kb
DNA ripetitivo	Un'ampia frazione, si veda la *Figura 7.1*	Pochissimo
Trascrizione	La maggioranza dei geni viene trascritta individualmente	Vengono trascritti più geni uno di seguito all'altro
Introni	Si trovano nella maggior parte dei geni	Assenti
% di DNA codificante	~ 3%	~ 93%
Utilizzazione dei codoni	Si veda la *Figura 1.22*	Si veda la *Figura 1.22*
Ricombinazione	Almeno una volta per ciascuna coppia di omologhi alla meiosi	Nessuna
Eredità	Mendeliana per le sequenze sull'X e sugli autosomi; patrilineare per le sequenze sull'Y	Esclusivamente matrilineare

Codice genetico	**Universale**	**Universale variato a 4 codoni**
Segregazione	**Mitosi e meiosi**	**Distribuzione casuale**
Tasso mutazione		**10 volte maggiore**



Conclusione: Gran parte delle cellule è eteroplasmica, anche se, casualmente, alcune possono ricevere un solo tipo di organello (per esempio tutti gli organelli normali o tutti mutanti).


Heteroplasmia
Affected
© Clinical Tools, Inc.


Homoplasmia
Affected
© Clinical Tools, Inc.

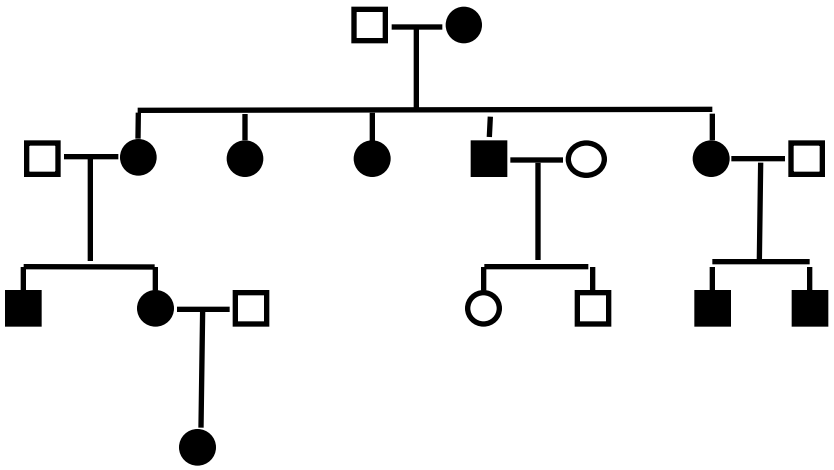
EREDITARIETA' MITOCONDRIALE

Mutazioni nel DNA mitocondriale (mtDNA)
Maschi e femmine sono affetti
Femmine trasmettono la malattia (i maschi non la trasmettono)

Variabilità nell'espressione dovuta alla quantità di mtDNA mutato

OMOPLASMIA (molecole wild type o mutate di mtDNA tutte uguali)
ETEROPLASMIA (popolazione mista di mtDNA normale e mutato)

EFFETTO SOGLIA: effetto delle mutazioni del mtDNA è determinato dalla relativa proporzione wild type / mutato in un determinato tessuto



Esclusione allelica: imprinting

A A

Entrambi gli alleli (geni autosomici) sono attivi (quasi tutti i loci)

X X

Solo un allele (geni X-linked) è attivo (quasi tutti i loci)

ESCLUSIONE ALLELICA: casuale

Inattivazione dell' X

Meccanismi sconosciuti

Non casuale:
GENOMIC IMPRINTING (origine parentale)
Può essere anche tessuto specifico

OSSERVAZIONI → IMPRINTING

Osservazioni sperimentali.

Embrioni di topo ottenuti per fusione di pronuclei di origine materna o paterno (diploidia uniparentale) non si sviluppano.

Osservazioni naturali.

Diploidia uniparentale paterna: Non sviluppa l'embrione e l'epitelio trofoblastico può trasformarsi in coriocarcinoma.

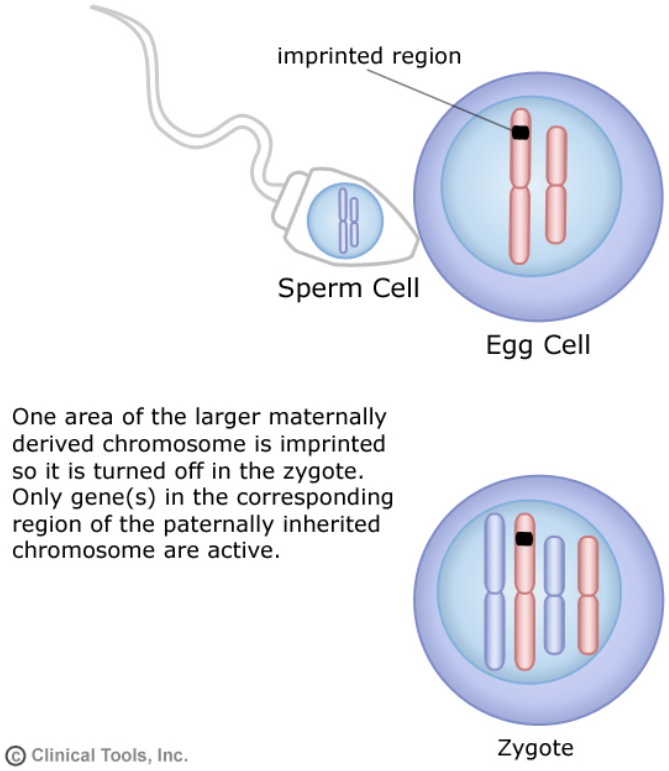
Diploidia uniparentale materna: Massa disorganizzata di tessuti embrionali (**"teratoma ovarico"**) senza presenza di annessi extra-embrionali.

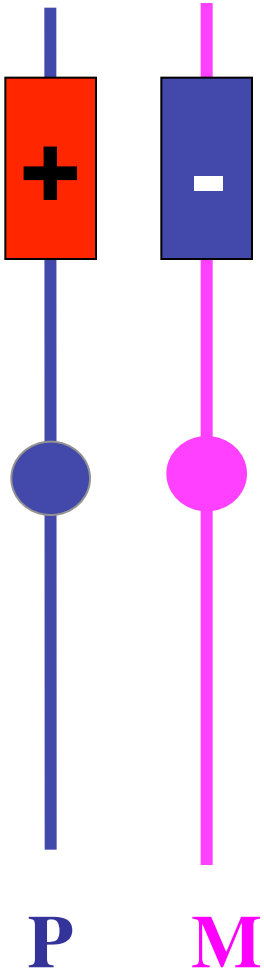
Fenotipi di aborti umani triploidi sono diversi se il contributo diploide è materno o paterno.

Alcuni caratteri autosomici dominanti si manifestano solo quando ereditati dal padre o dalla madre.

IMPRINTING GENOMICO

Origine parentale dei cromosomi: effetto sull'espressione dei geni

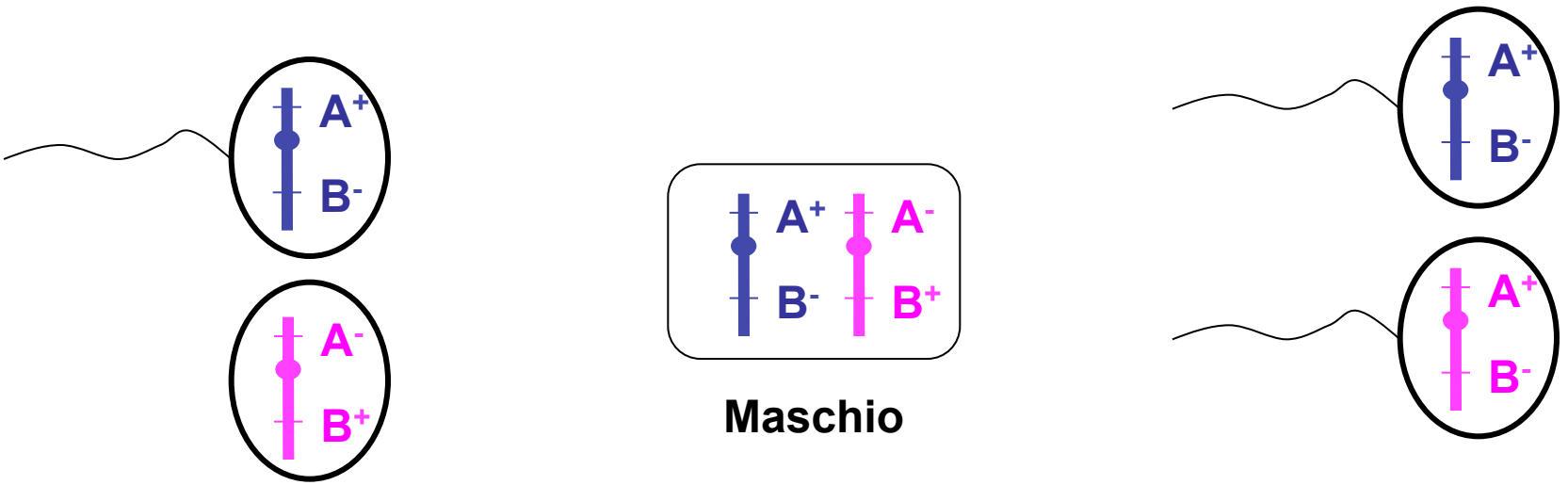




Fecondazione

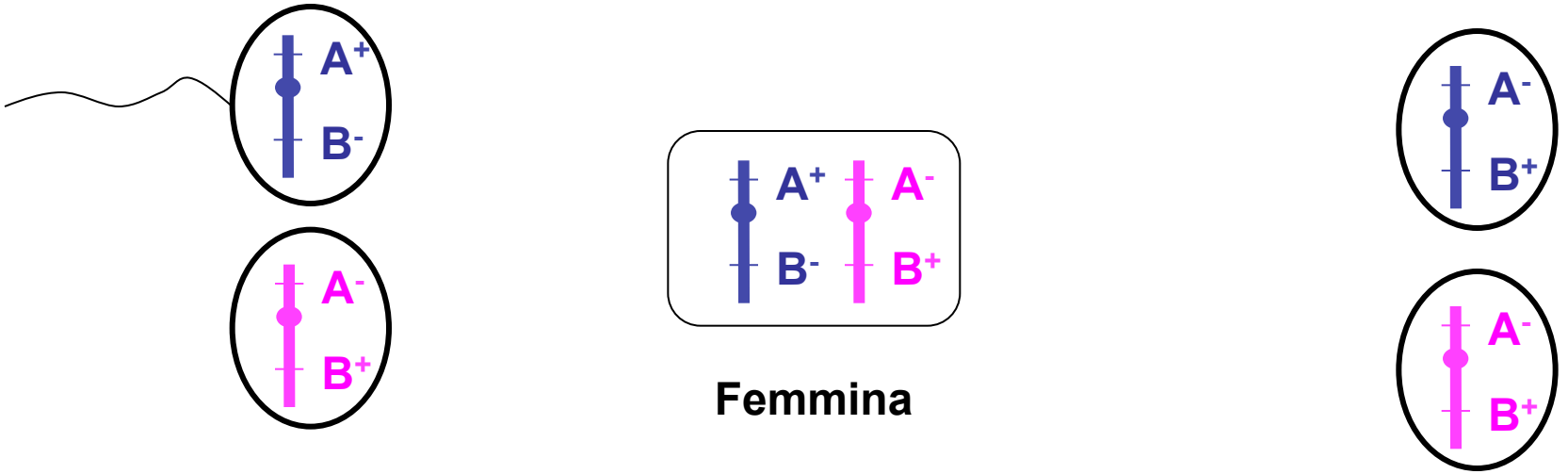
Gametogenesi

Gameti → Zigote Cellule somatiche → Gameti



Maschio

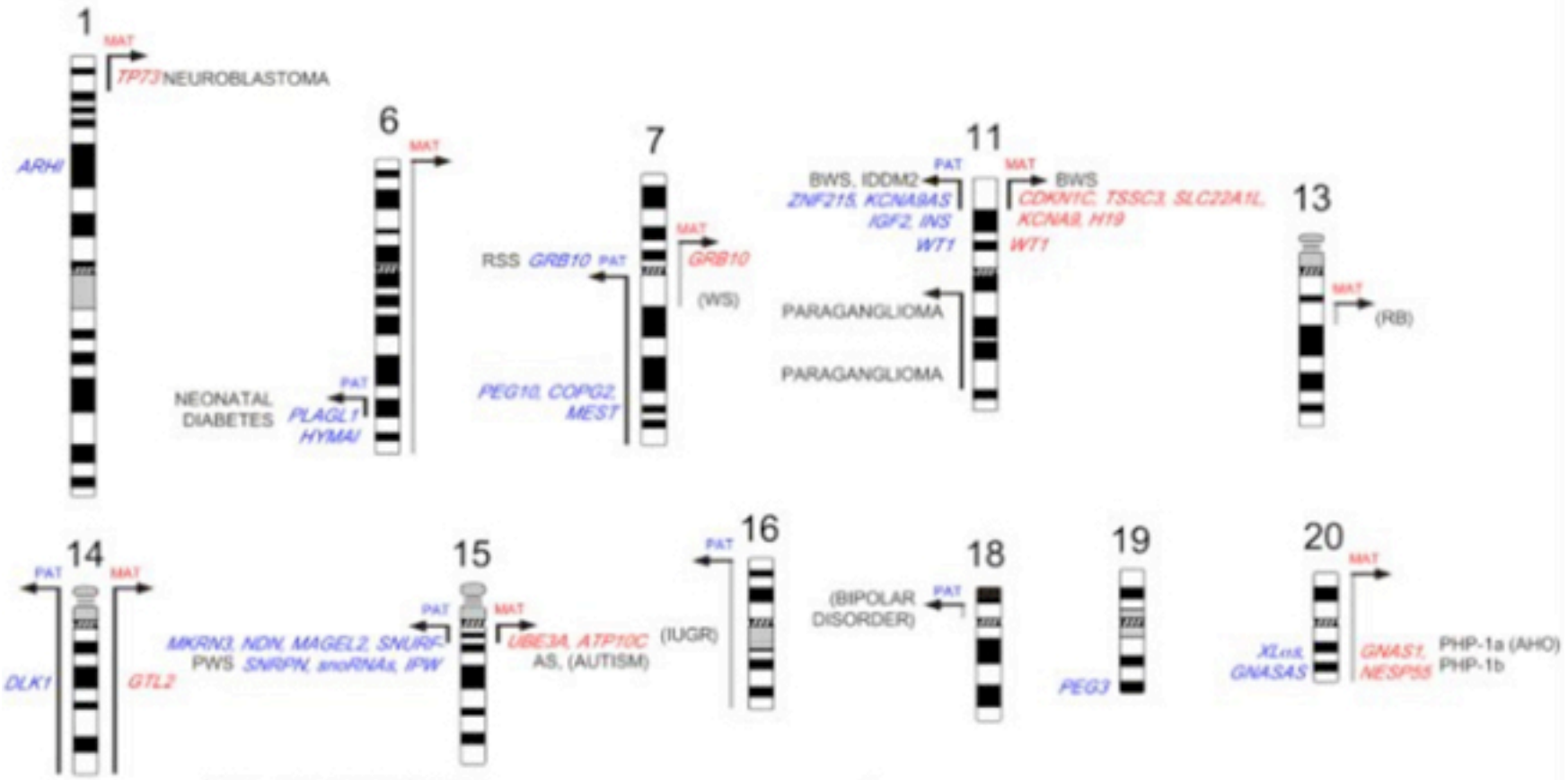
Rimozione vecchio e definizione nuovo imprinting



Femmina

Human imprinting map

http://greallyoffice.aecom.yu.edu/



RSS: Russell-Silver syndrome
WS: Williams syndrome
BWS: Beckwith-Wiedemann syndrome
IDDM: Insulin-dependent diabetes mellitus
RB: Retinoblastoma
PWS: Prader-Willi syndrome
AS: Angelman syndrome
IUGR: Intrauterine growth retardation
PHP: Pseudohypoparathyroidism

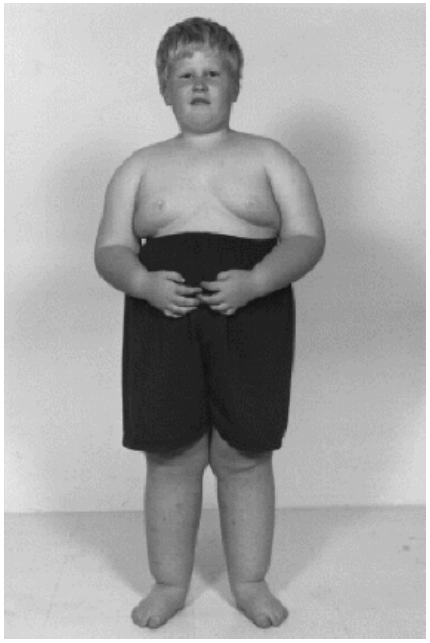
| Imprinted phenotypic effect clear

| Possible Imprinted phenotypic effect

PAT ← Evidence for paternally expressed gene(s)

MAT → Evidence for maternally expressed gene(s)

Prader-Willi syndrome



Obesità
Ritardo mentale
Problemi linguaggio
Mani e piedi piccoli
Ipogonadismo

Angelman syndrome (Happy Puppet)

Grave ritardo mentale
Assenza della parola
Lingua protrudente
Atassia e movimenti stereotipati
Crisi epilettiche

Entrambe le malattie possono essere dovute a:

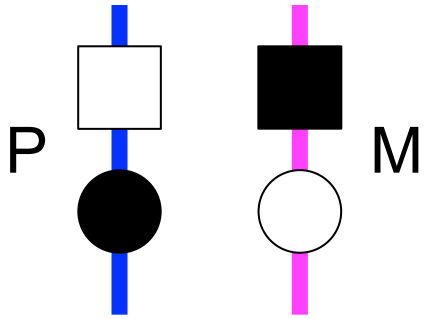
1. Delezione dell'intera regione cromosomica 15**q11-13** (paterna nella PWS, materna nella AS);

2. Disomia uniparentale (UPD) (materna nella PWS, paterna nella AS);

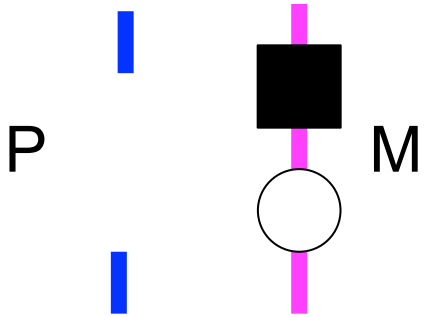
3. Errore di imprinting

4. Solo per la sindrome di Angelman: mutazione nella copia materna del gene AS

Cause di Prader-Willi/Angelman: **delezione**

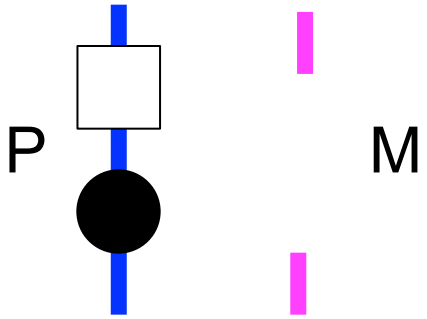


Pattern di espressione normale: espresse (simbolo bianco) le regione PWS (quadrato) del cromosoma paterno e AS (cerchio) del cromosoma materno



Delezione sul cromosoma paterno

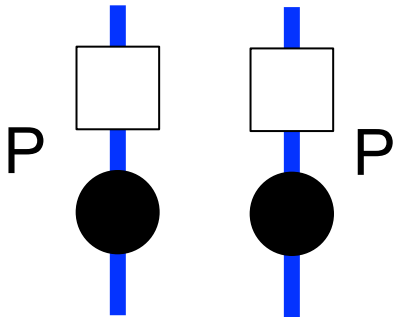
Sindrome di Prader-Willi: manca espressione della regione PWS



Delezione sul cromosoma materno

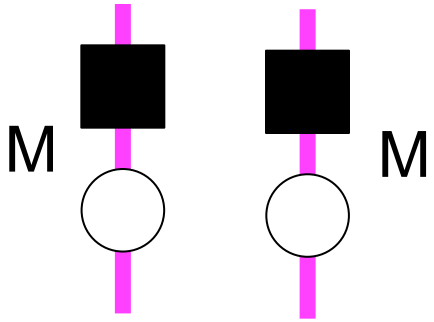
Sindrome di Angelman: manca espressione della regione AS

Cause di Prader-Willi/Angelman: **disomia uniparentale (UPD)**



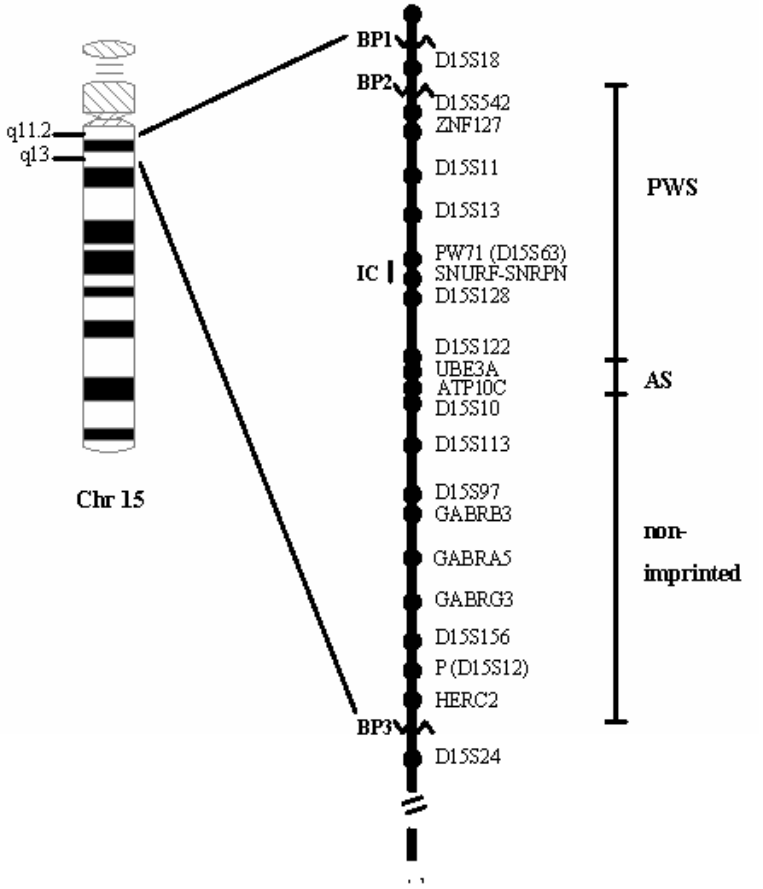
UPD paterna

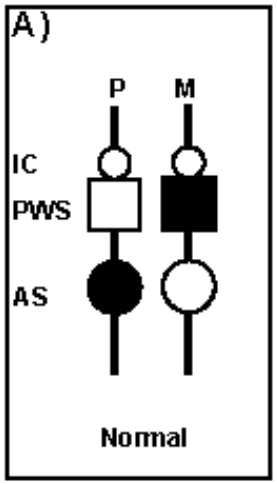
Sindrome di Angelman: assenza espressione regione AS

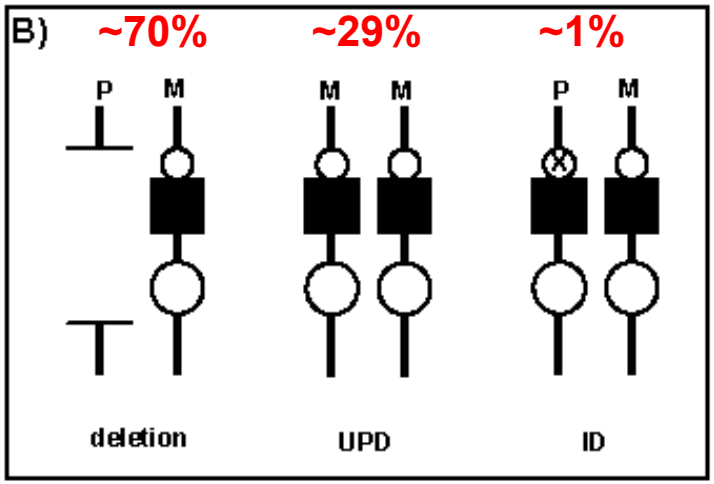


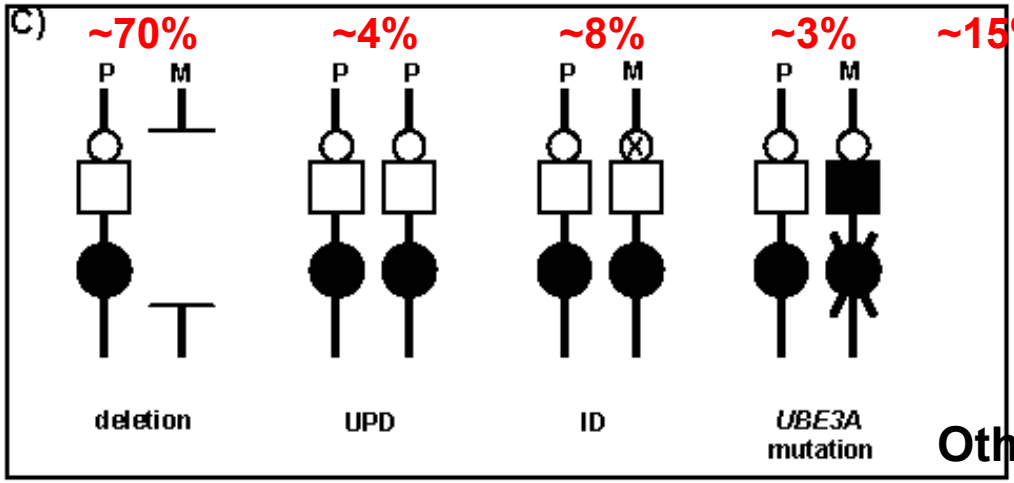
UPD materna

Sindrome di Prader-Willi: assenza espressione regione PWS



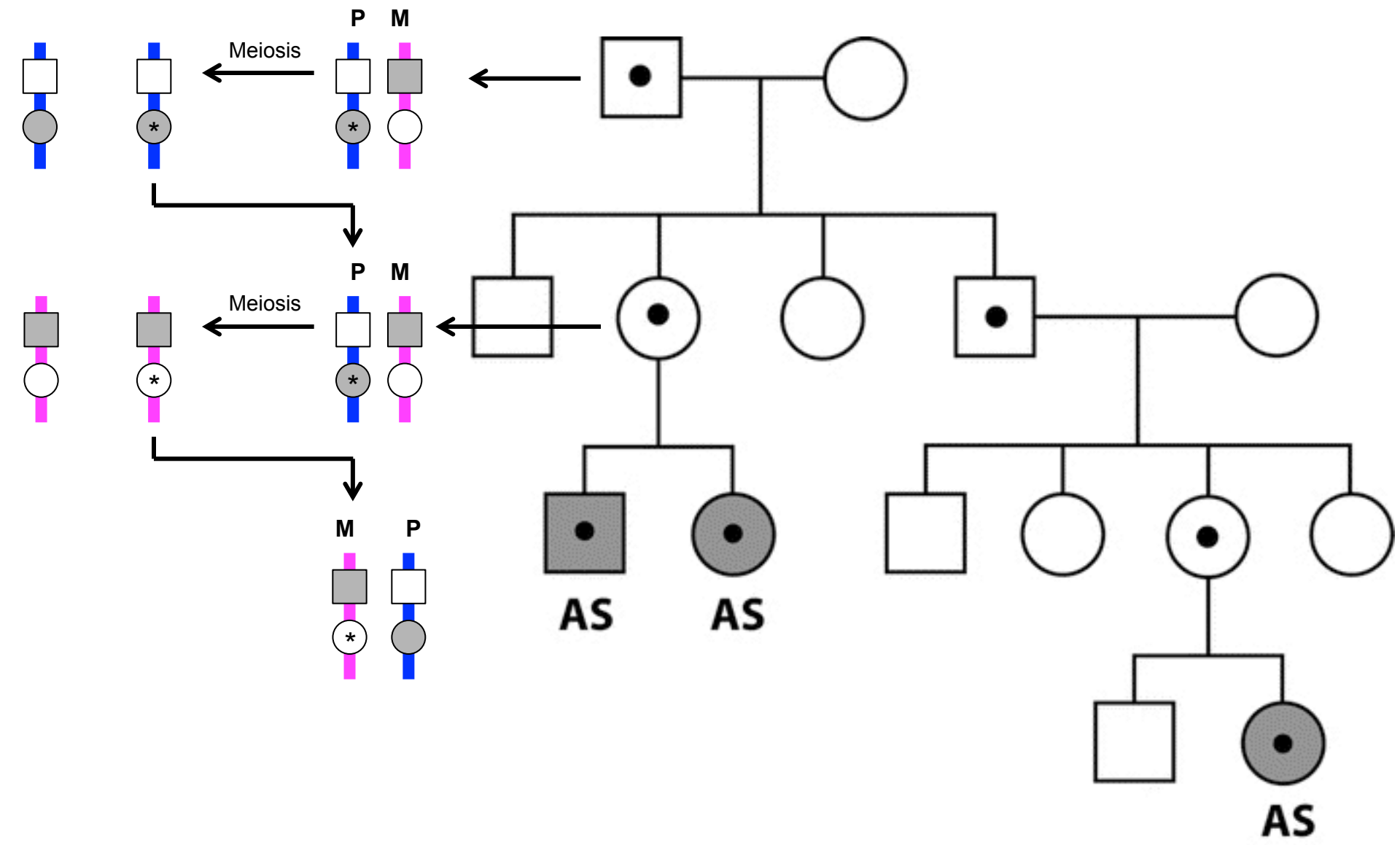






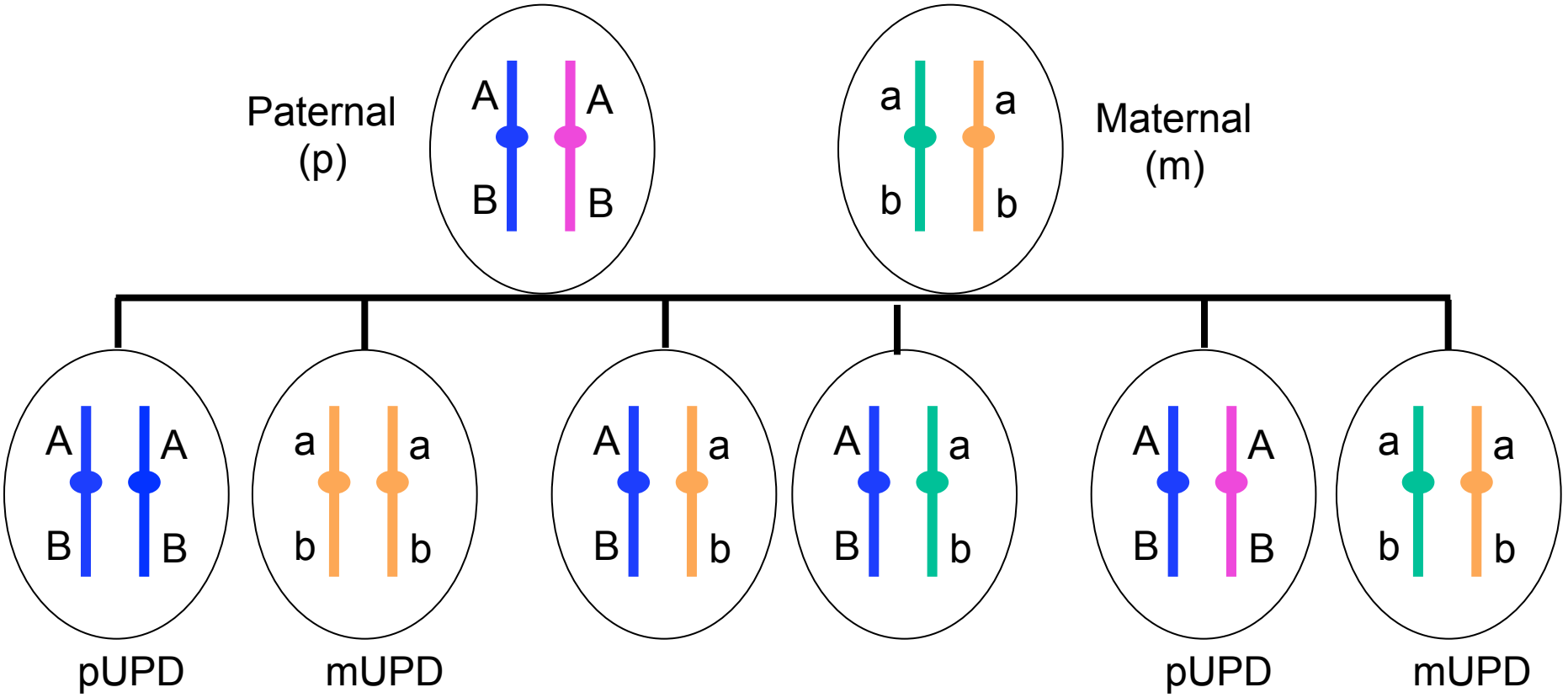
B) PWS
C) AS

ID: imprinting defect
IC: Imprinting center

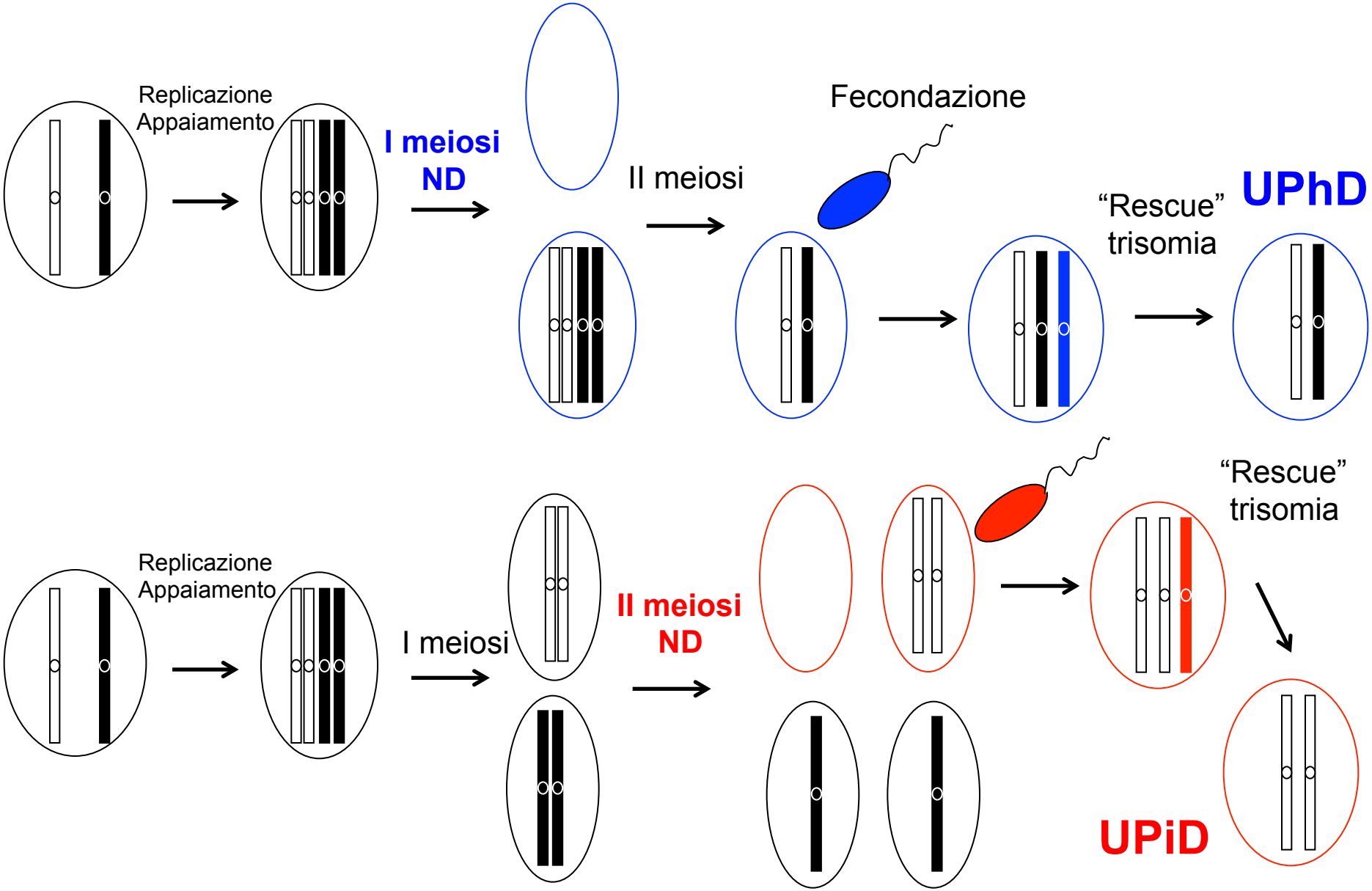

P
M
Meiosis
P
M
Meiosis
M
P
AS
AS
AS
● = UBE3A mutation or IC deletion carrier
= Angelman syndrome

Uniparental disomy (UPD): definition

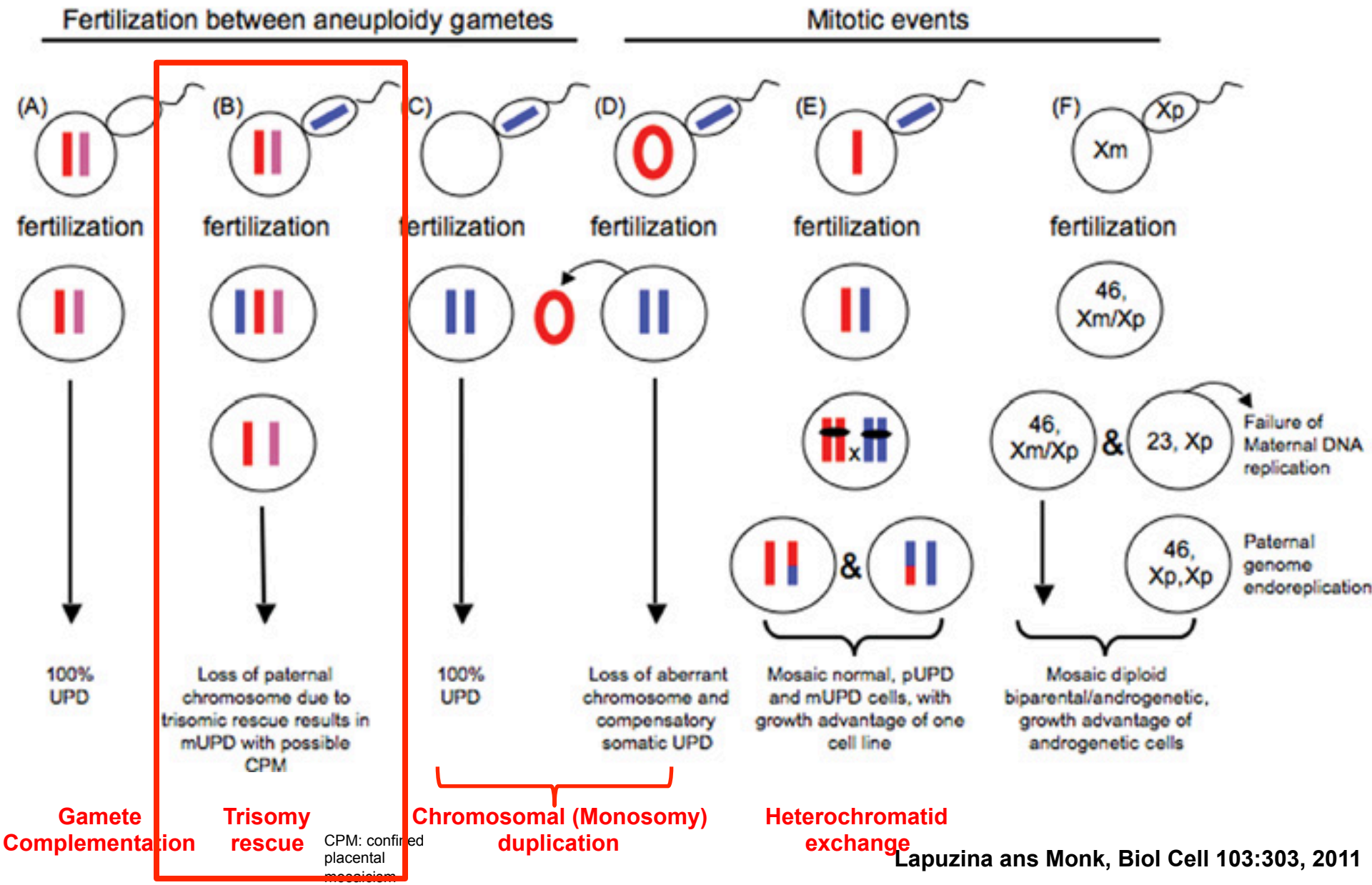
- Correct chromosome number
- Both homologs of a specific chromosome (or chromosomal segment) are inherited from the same parent



Cause UPD:
non disgiunzione in meiosi e “rescue della trisomia



Uniparental disomy (UPD): causes



CPM: confined placental mosaicism

Lapuzina ans Monk, Biol Cell 103:303, 2011

Uniparental disomy (UPD): causes
Errors in meiosis and mitosis

- **Trisomic rescue.** Chromosome loss in trisomy to generate a disomic foetus (1/3 of cases). This is the most common mechanism for UPD.

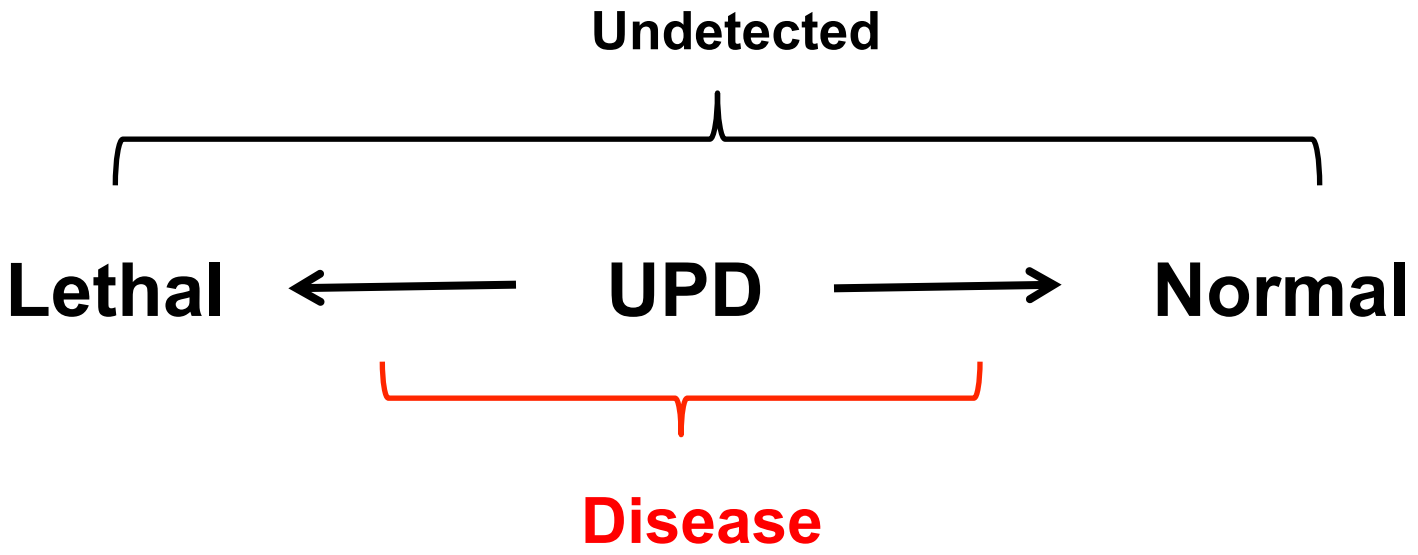
- **Gamete complementation.** Fertilization of gametes, both abnormal, one disomic and the other nullisomic. One parent has contributed both homologs to the zygote and the other parent has contributed none.

- **Chromosomal duplication**. Fertilization of a normal with a nullisomic gamete and duplication of the chromosome, always leading to isodisomy. Or rare mitotic attempt to correct aneuploidy with loss of an aberrant homologue of a chromosome pair (pseudodiploid) and chromosomal duplication

- **Heterochromatid exchange**. Somatic recombination leading to segmental regions of isodisomy between the point of recombination and the telomere in the daughter cells

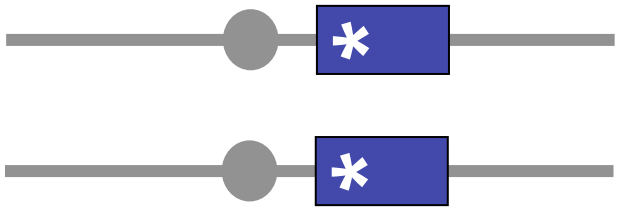
- **Gene conversion**. Small (330-1000 bp) isolated regions of isodisomy

Uniparental disomy (UPD): consequences

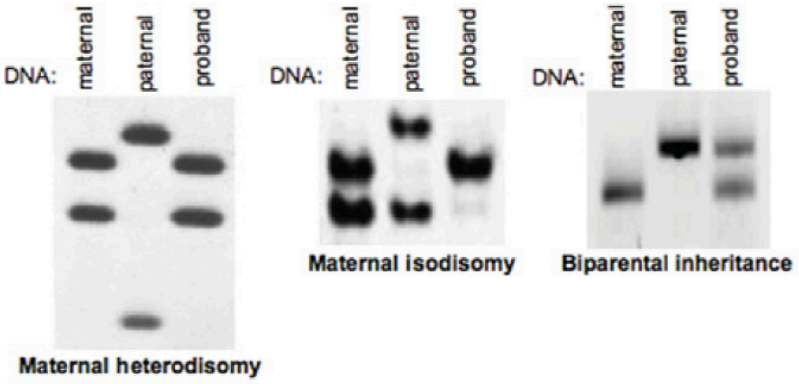


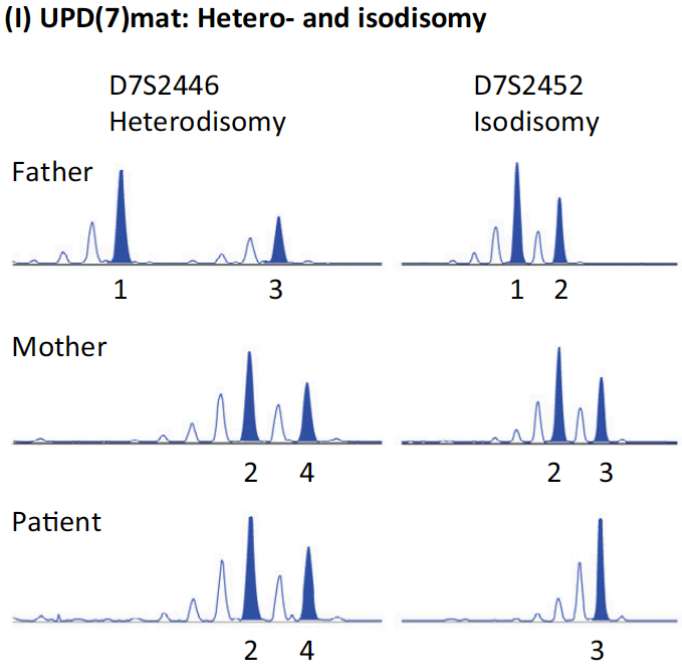
Altered imprinted gene dosage (iso- and hetero-disomy)

Unmasking mutant (*)recessive mutations (isodisomy)



Uniparental disomy (UPD): identification
marker genotyping



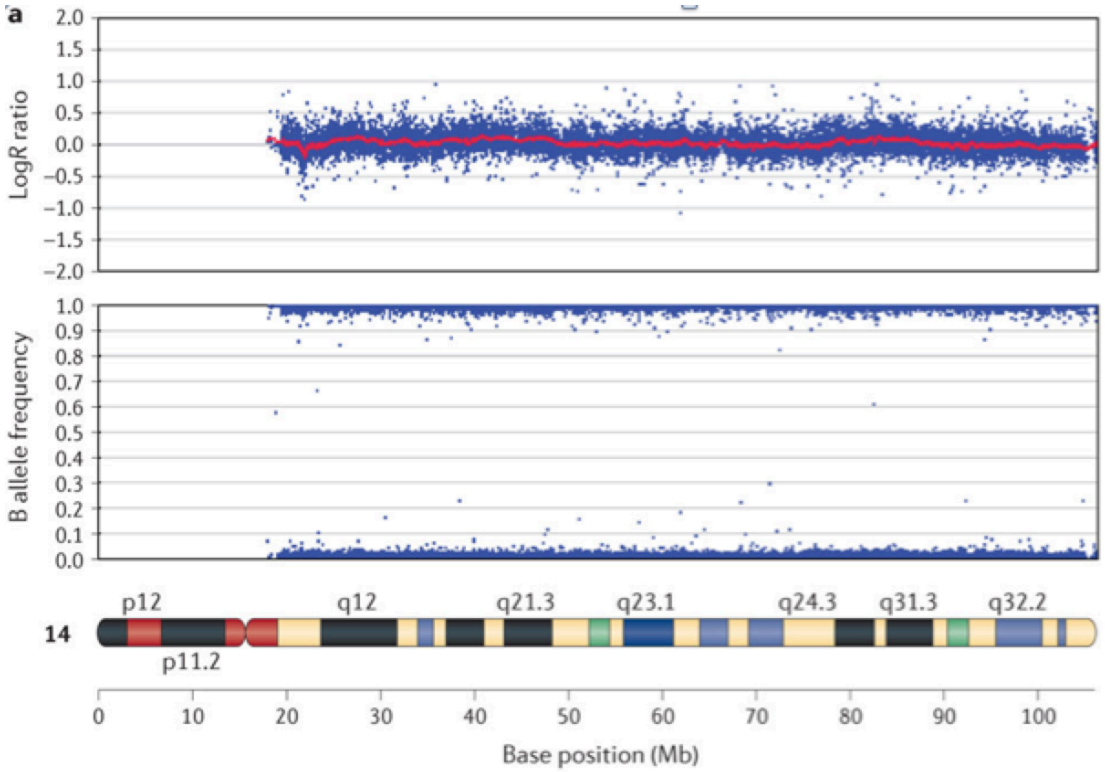


Tetranucleotide repeats

Dinucleotide repeats

Uniparental disomy (UPD): identification

SNP array



Normal copy number

Allele B

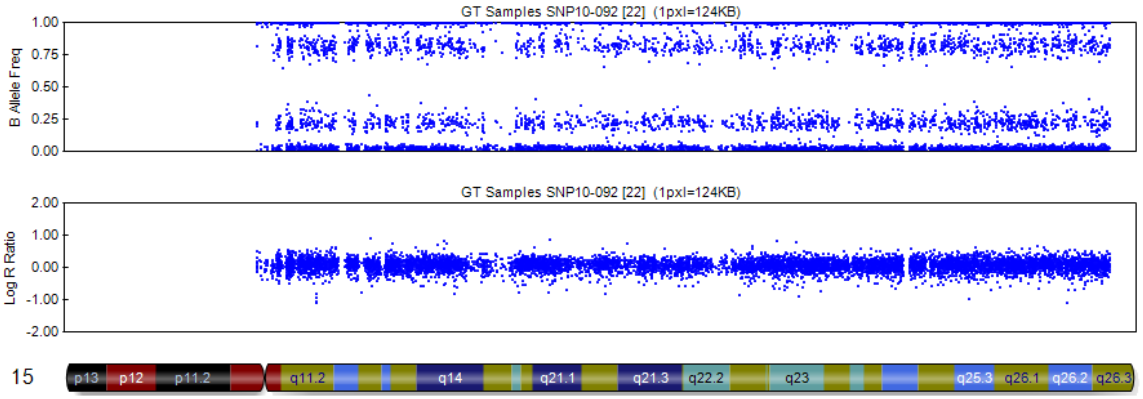
Lack of heterozygosity

Allele A

Identification of only isodisomy

Unless parents' DNA is available for comparison

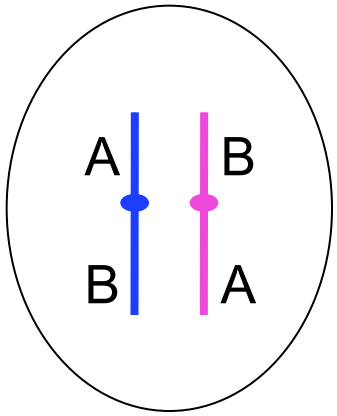
Uniparental disomy (UPD): detection of mosaicism

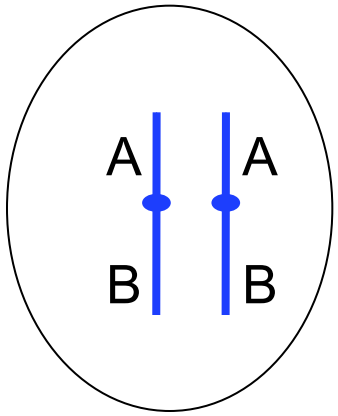


0.9

0.1

20%





80%

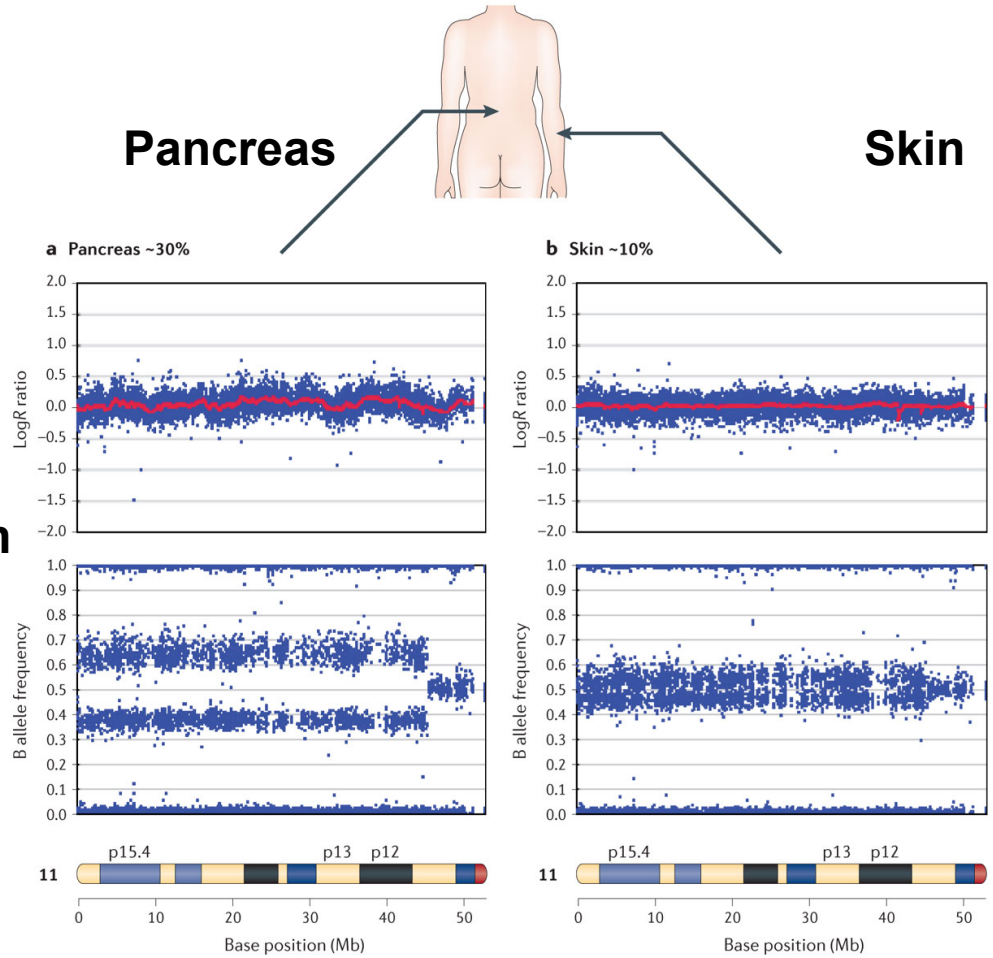
BAF Locus 1	1/2x0.2=0.1	0/2x0.8=0	0.1 > 0
BAF Locus 2	1/2x0.2=0.1	2/2x0.8=0.8	0.9 < 1

Total

Segmental uniparental disomy (UPD)

Pancreas

Skin

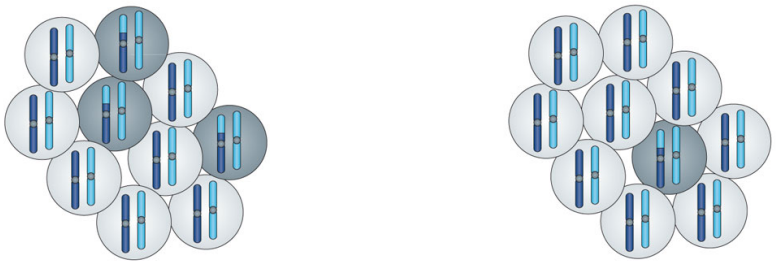


30% mosaicism

0.65

0.35

10% mosaicism



Nature Reviews | Genetics

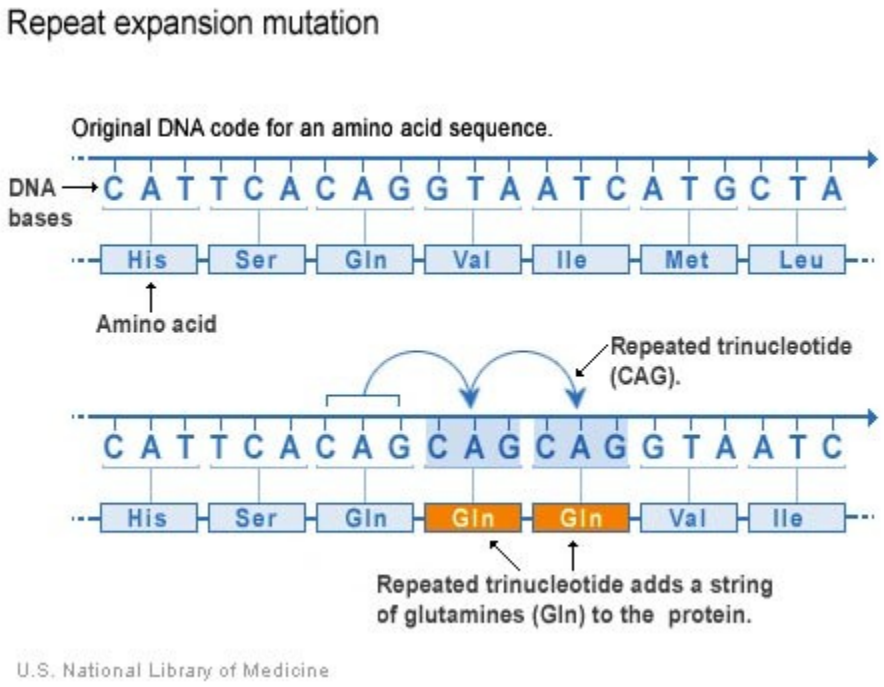
Dynamic mutations

STABLE MUTATIONS
from generation to generation

All affected members of a family
share the identical inherited mutation

Unstable expanding repeats
(DYNAMIC MUTATIONS)

Microsatellite: 1-6 nt



Unstable - dynamic mutations

Type: Usually trinucleotide, but even 4 (x1), 5 (X2), 6 (x2) or 12 (x1) nt, repeat expansion. It becames a mutation when the number of triplets is greater than in a normal gene (threshold)

Instability: Beyond the threshold the number of triplets in the disease gene continues to increase (from 20-30 to 3000-5000) as the gene is transmitted

Consequences on gene function: the growing triplet tract alters gene expression and/or function: faulty protein, splicing defects, suppression of expression, defective antisense regulation

Consequences on phenotype: Severe neuromuscolar and neudegenerative disorders

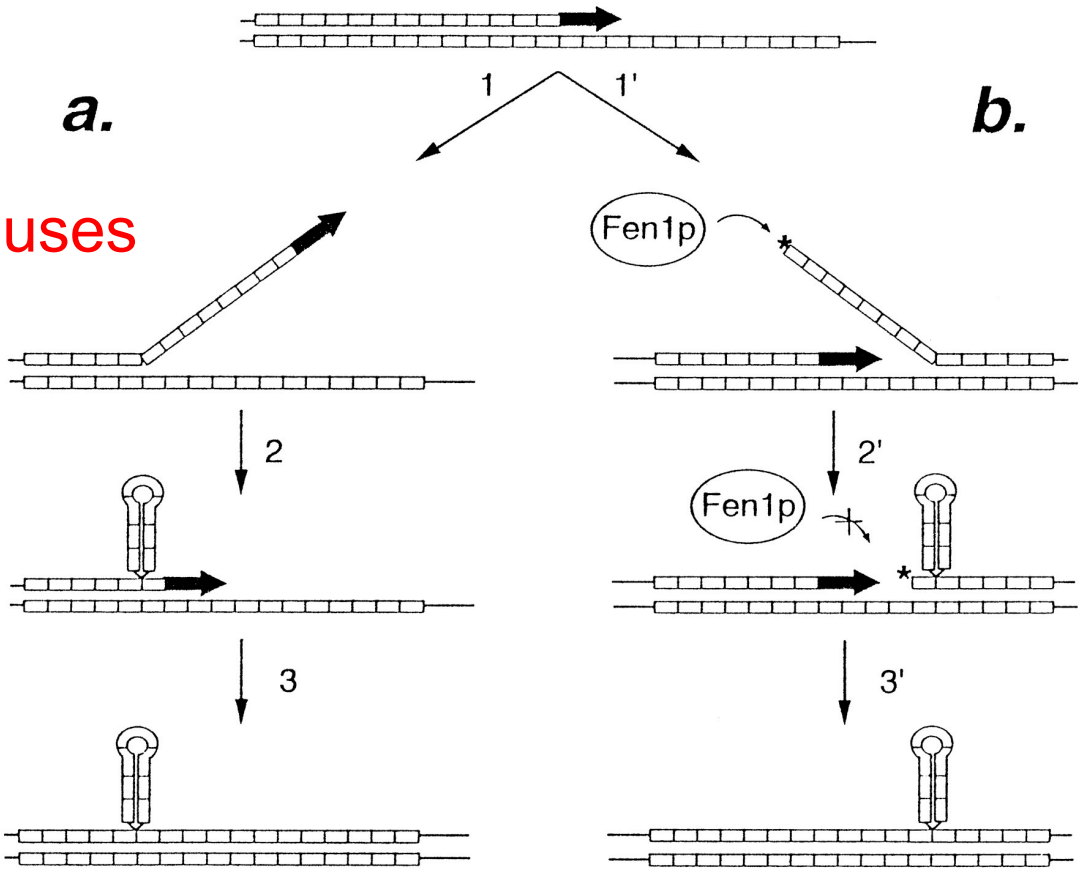
Causes of expansion

DNA metabolic processes, such as replication, repair, recombination

Replication associated causes

a) Replication slippage (small expansion)

b) Hairpin formation on Okazaki fragments (large expansion)

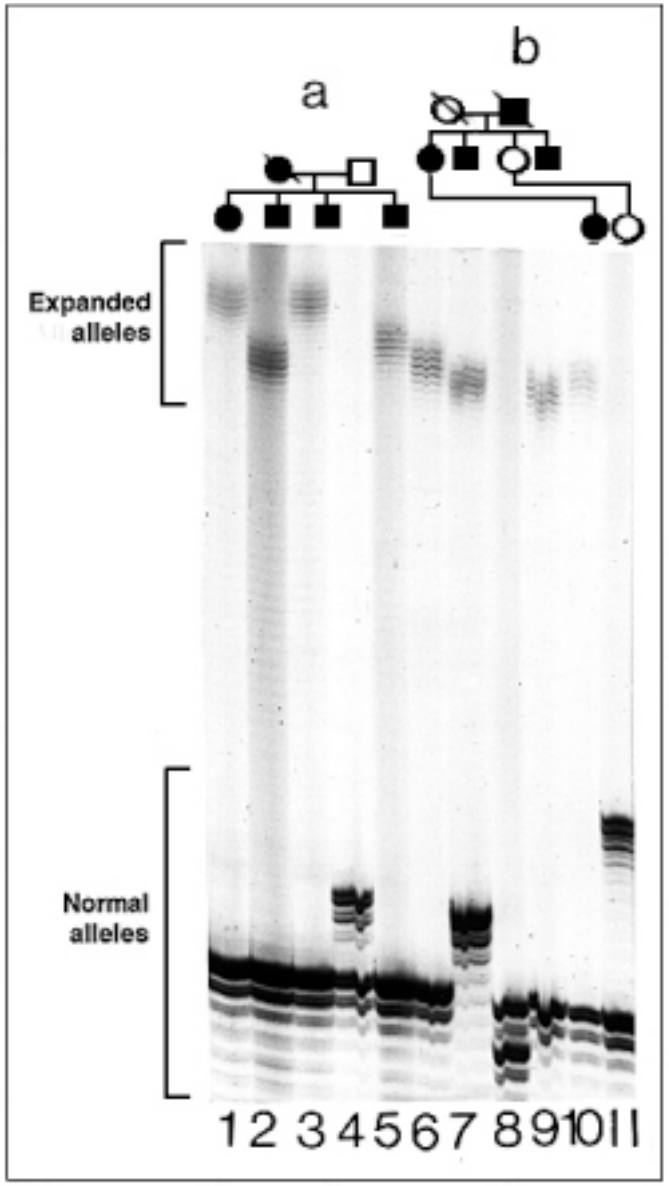


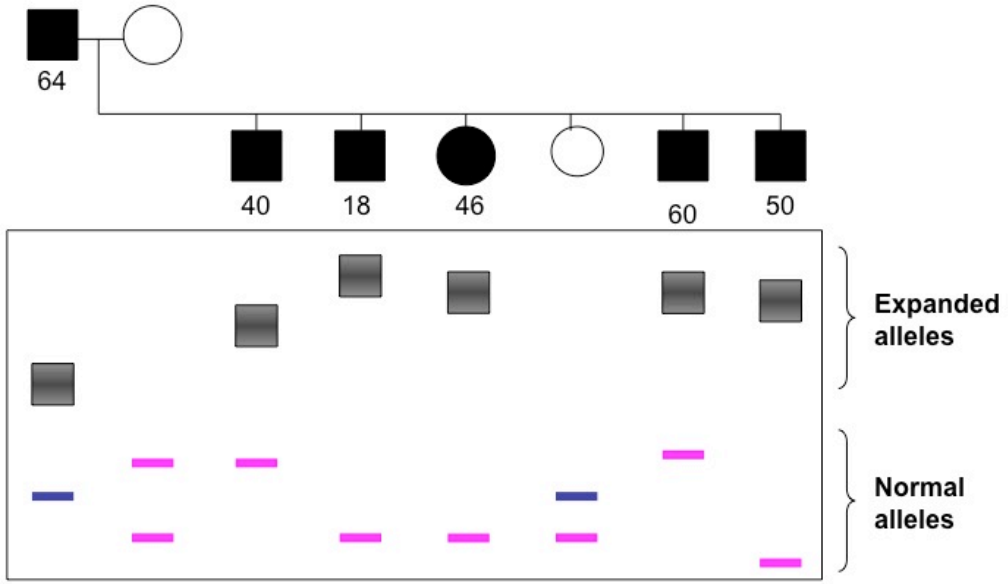
FEN1: endonuclease that removes 5' overhanging "flaps" of Okazaki fragments of the lagging strand

Expansion and anticipation

Severity and age of onset correlate with repeat number that tend to expand through generations

Heterogeneity of repeat length associated with somatic expansion





Classification and pathogenic mechanisms

Class A: Outside coding regions. Large expansion in introns, 5’- and 3’-UTR, promoter regions

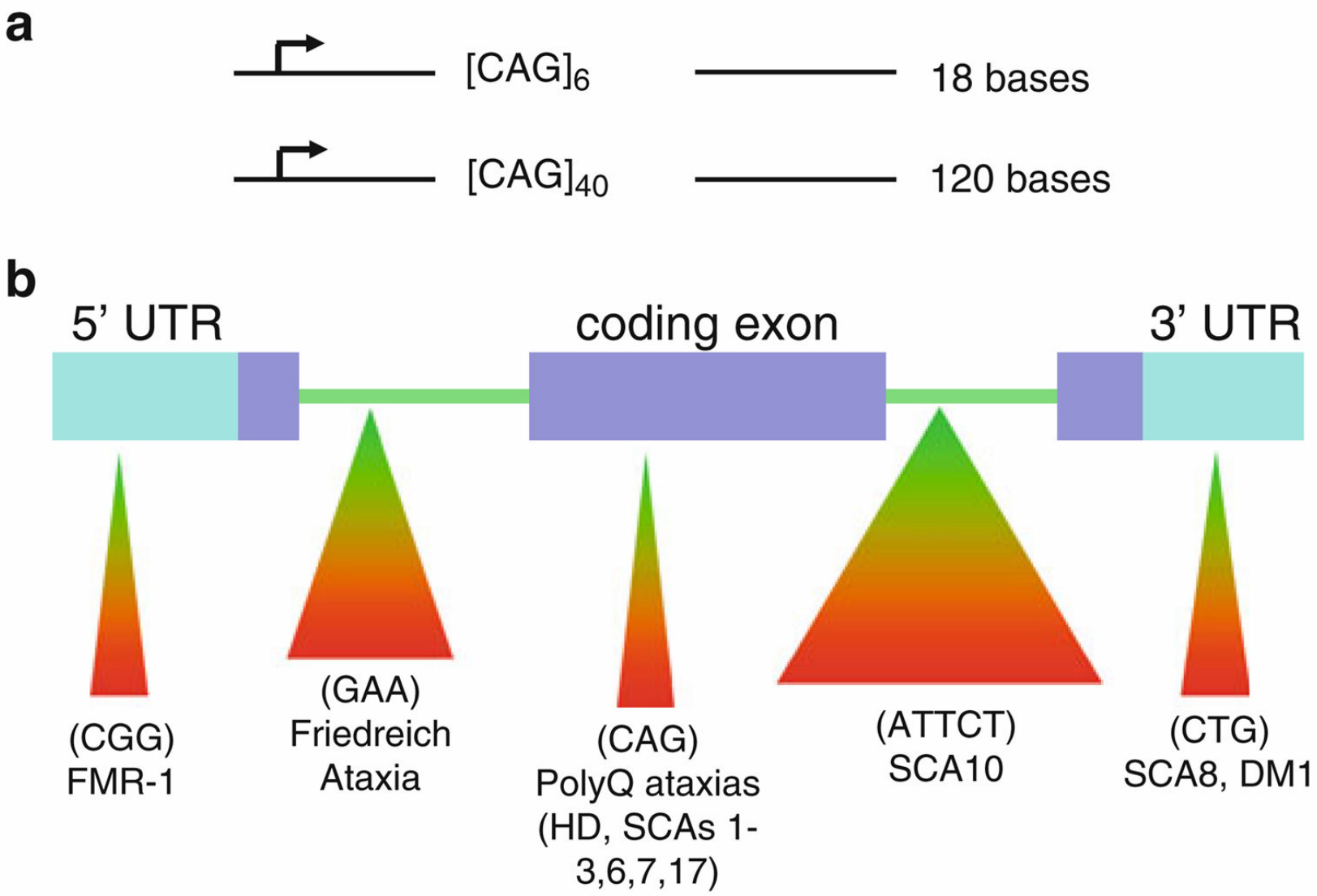
- Fragile X syndrome
- Myotonic dystrophy
- Friedreich ataxia

Class B: Limited expansion of the instable CAG triplet in coding regions (polyGlutamine/Q)

- Huntington’s disease
- Spinocerebellar ataxia (different forms)
- Kennedy disease

Class C: limited expansion of relatively stable imperfect (less dynamic) triplets GCN (polyAlanine/A)

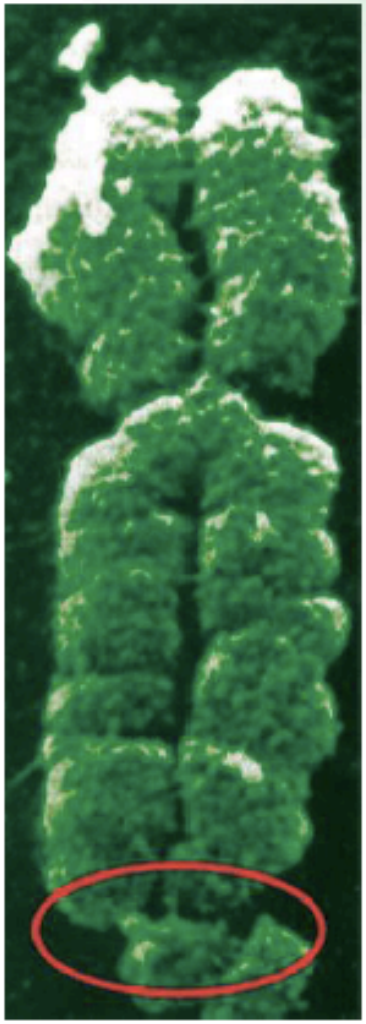
- Congenital central hypoventilation syndrome, or Ondine's curse



Spinocerebellar ataxia (SCA)
Myotonic dystrophy (MD)
Fragile X mental retardation (FMR)
Polyglutamine diseases (PolyQ)

Fragile X Syndrome

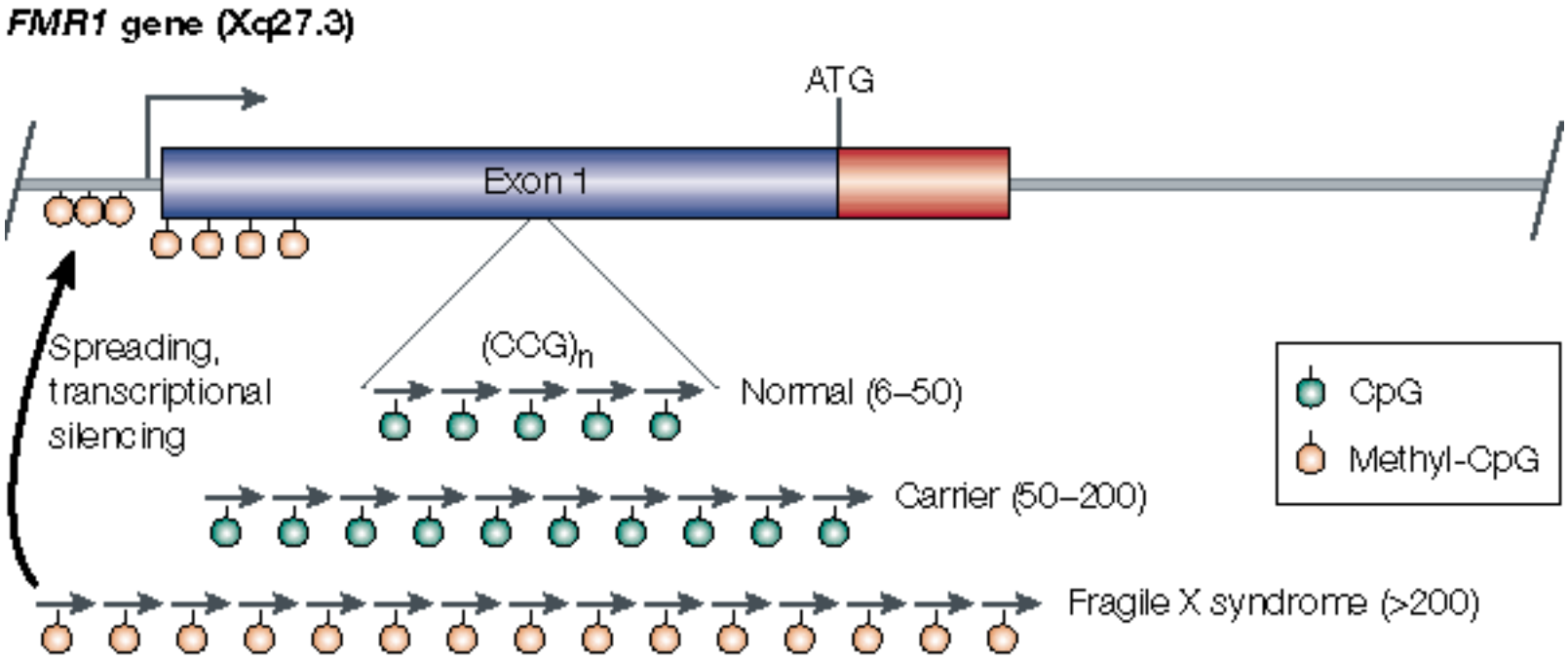
- **Fragile X Syndrome (FXS)** is caused by expansion of CGG repeats in the *FMR1* gene. It is the leading cause of hereditary mental retardation
- Fragile X carriers may be at risk for fragile X-associated tremor/ataxia syndrome (FXTAS), a form of age-related cognitive and motor dysfunction, or primary ovarian insufficiency (FXPOI), a cause of premature menopause.
- FXS and other *FMR1* disorders impact an estimated 1.5 million individuals in the US, and affect a broad range of populations and ages.
- New therapies and targeted drugs for treating FXS are in development.



FMR1 gene

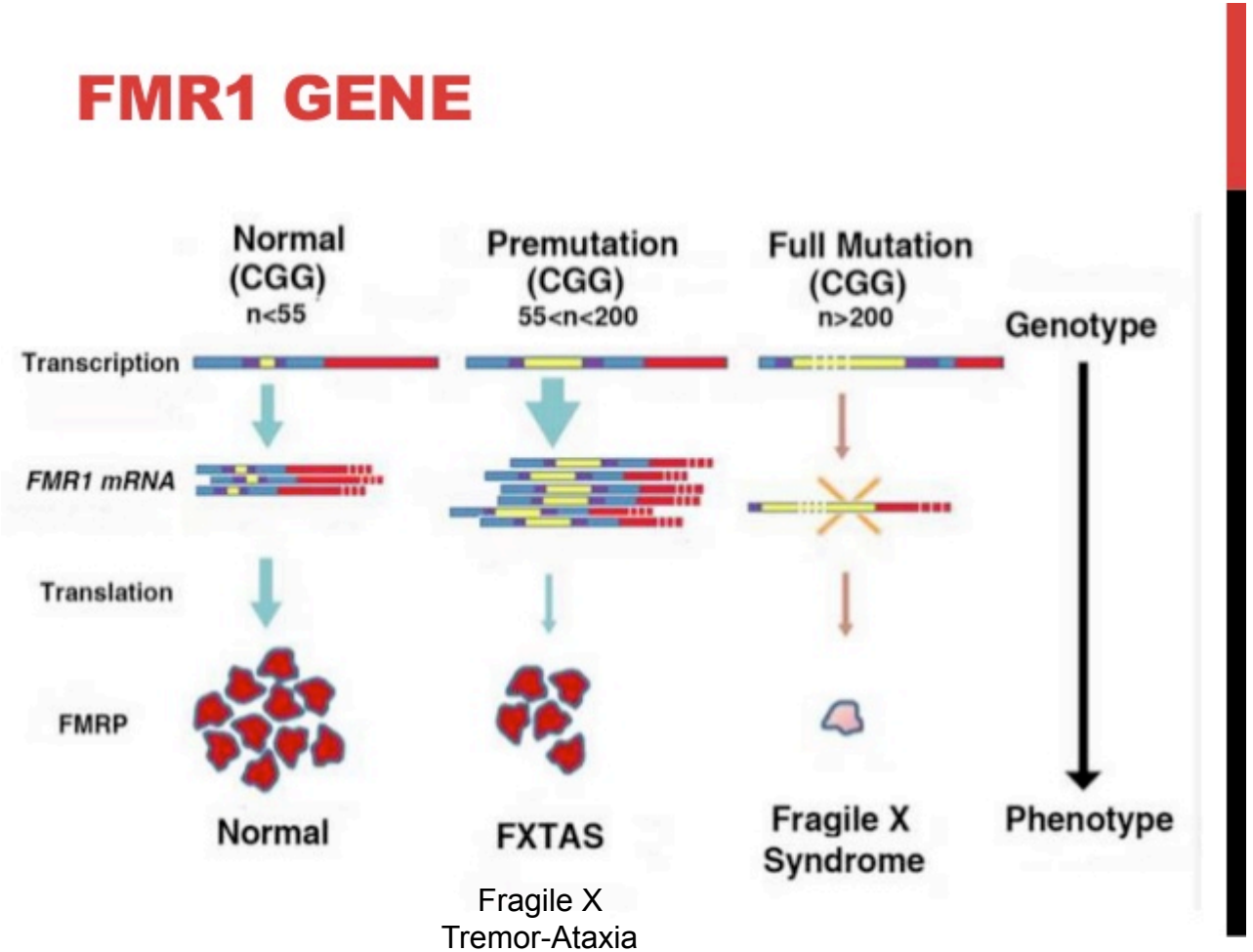
Xq27.3

Fragile X syndrome: gene FMR1



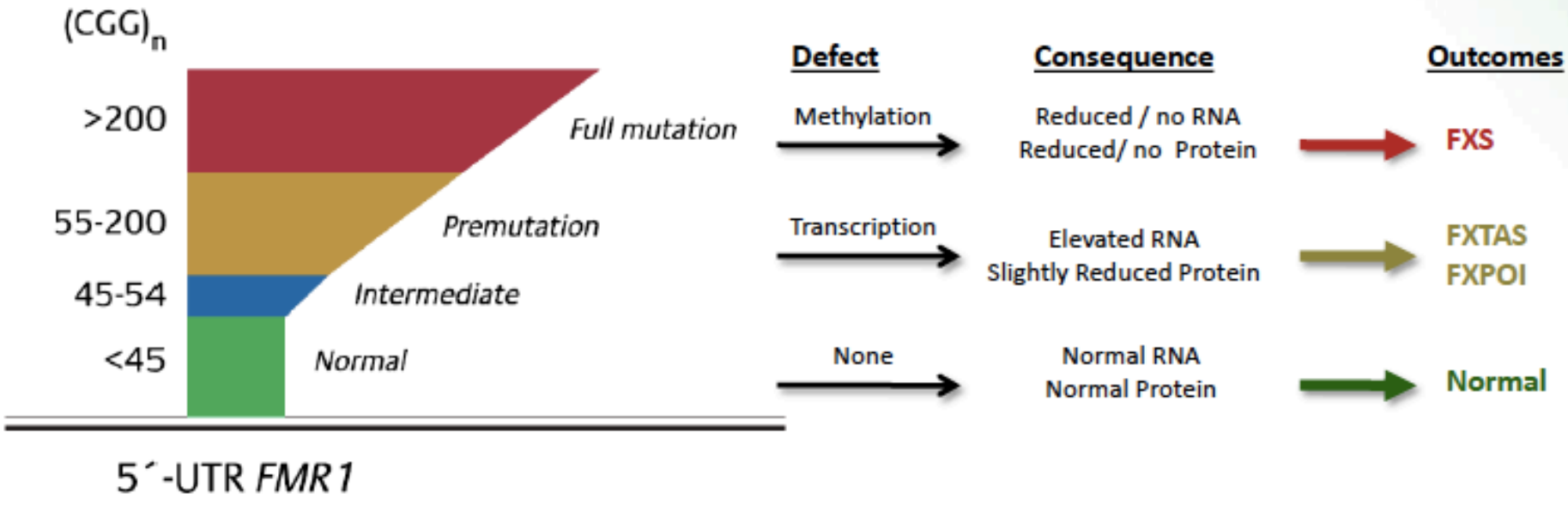
Nature Reviews | Genetics

Model of reduction of the FMR-1 protein leading to Fragile X syndrome



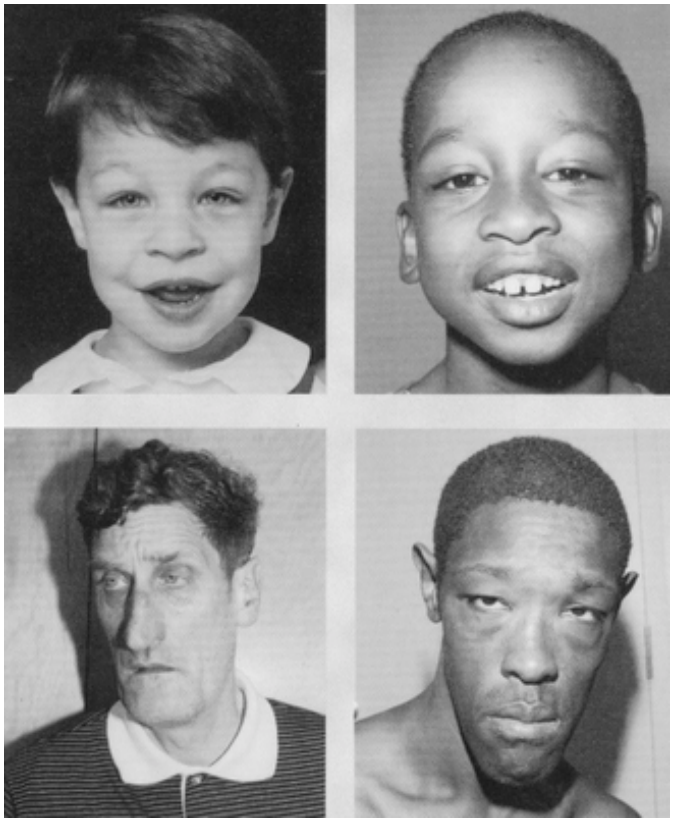
Fragile X Tremor-Ataxia Syndrome

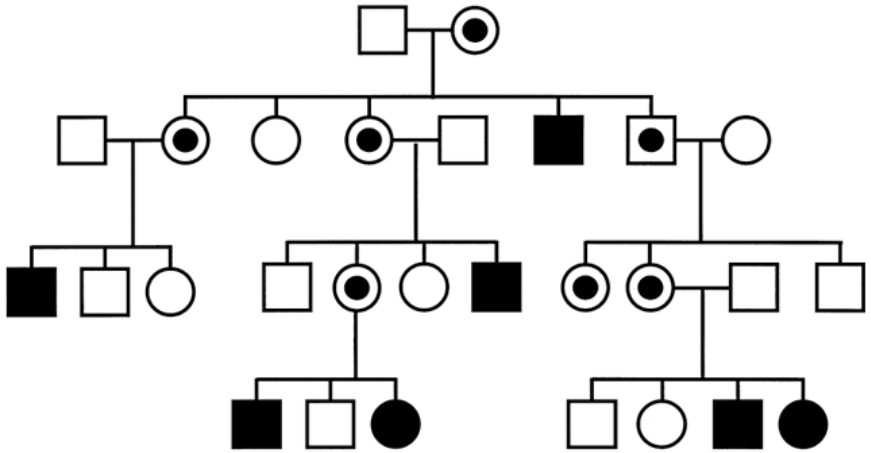
Fragile X Molecular Analyses



Expansion of CGG repeats and methylation status of the *FMR1* gene are used to study FXS and related disorders. PCR and Southern blot are the techniques applied

X FRAGILE
(una delle cause più frequenti di ritardo mentale moderato nei maschi)



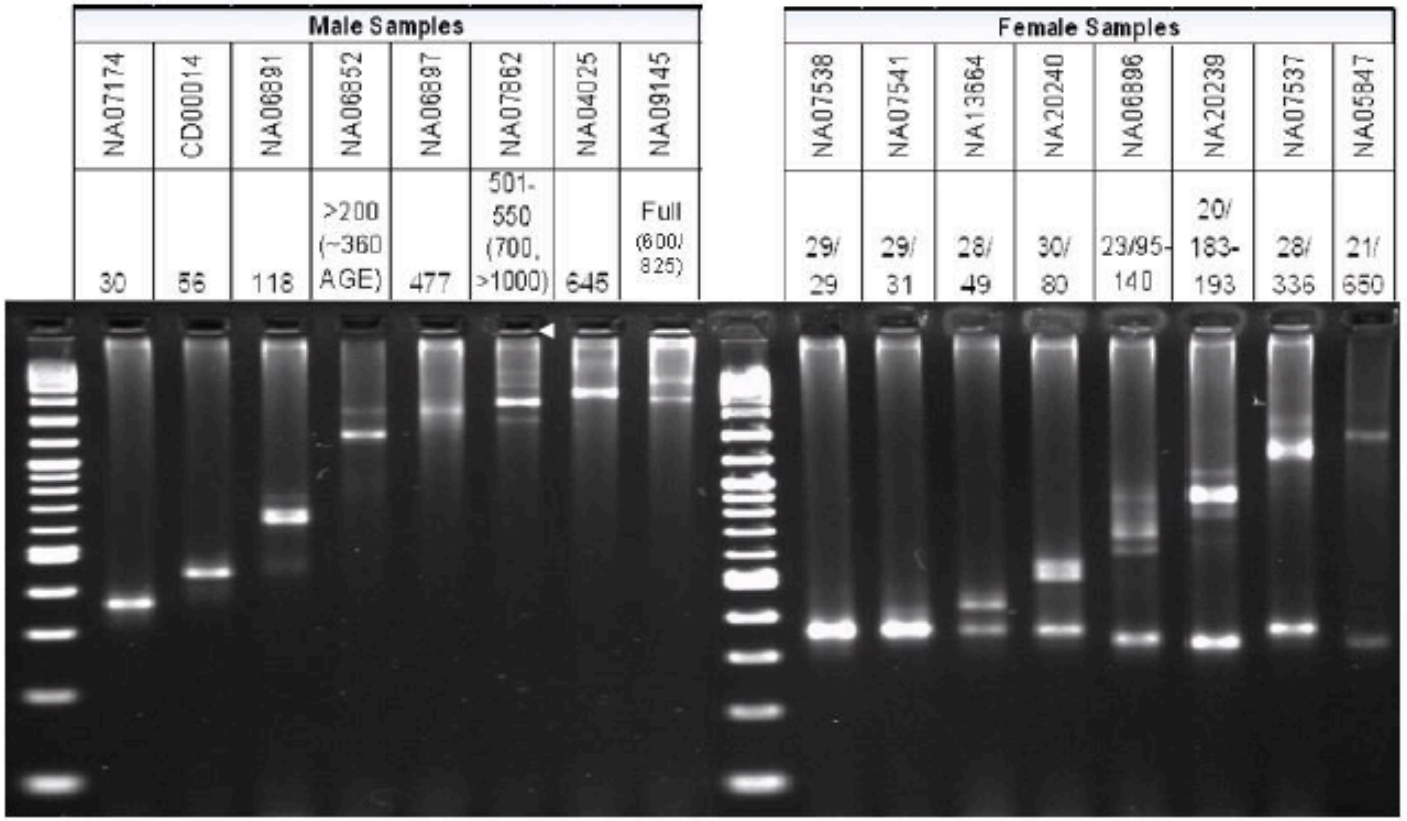


FMR1 Reverse Primer

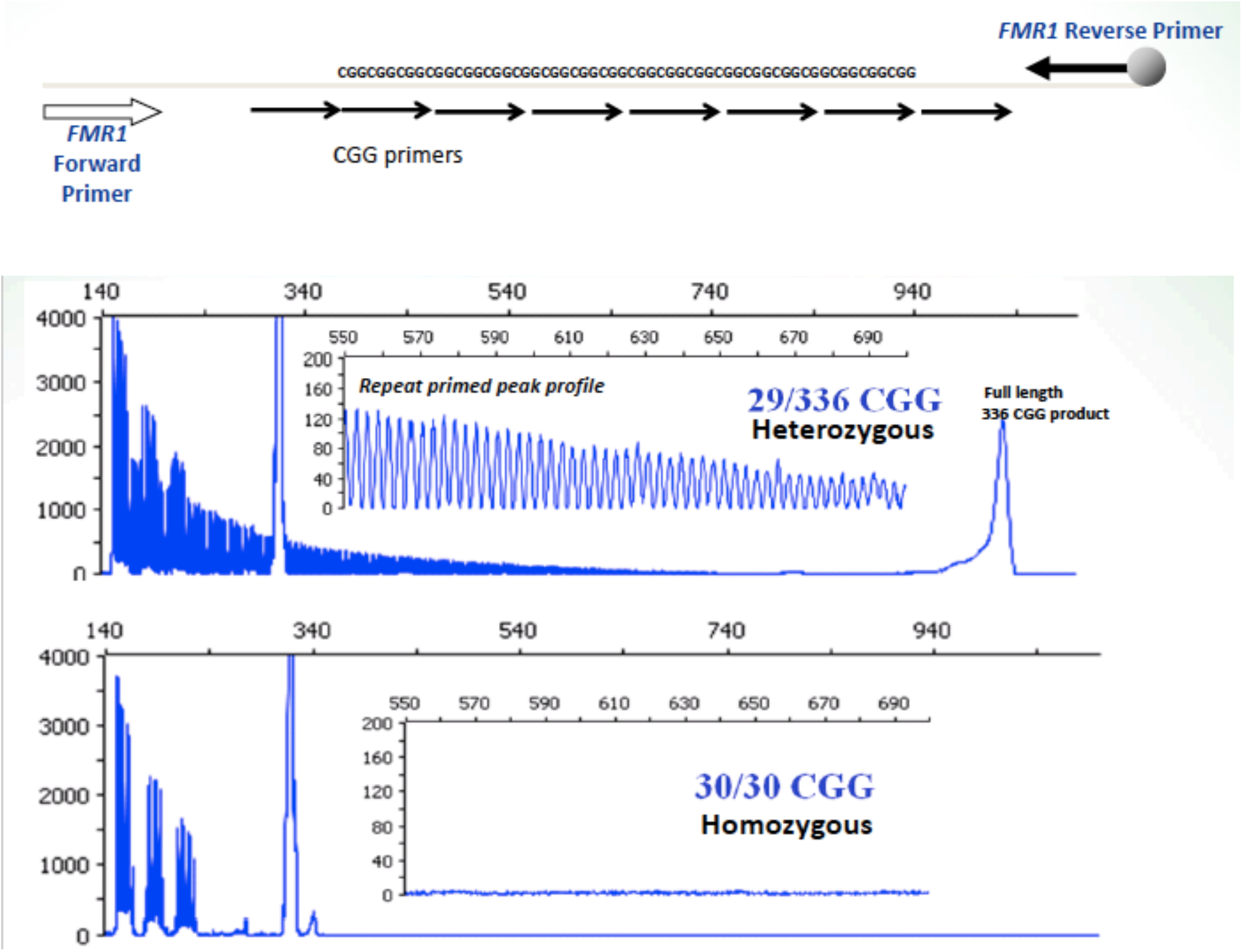
CGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG

FMR1 Forward Primer

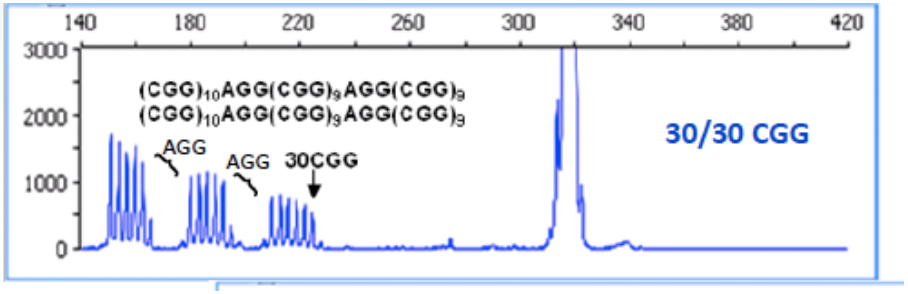
Amplification of grater than 1000 CGG repeats

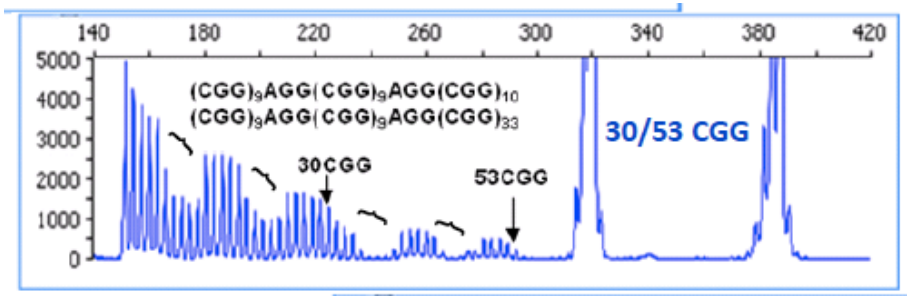


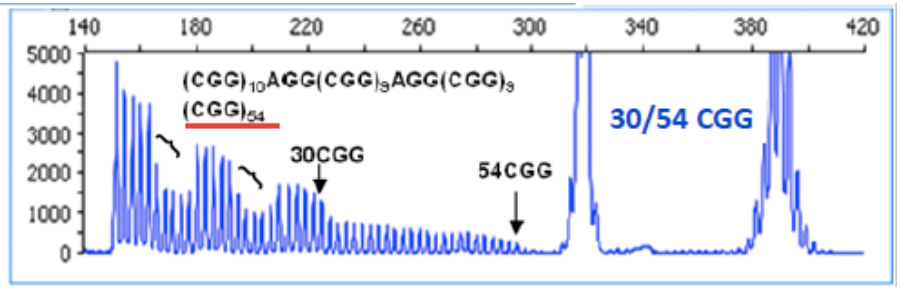
AmplideX *FMR1*


FMR1 Reverse Primer
CGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
FMR1 Forward Primer
CGG primers
140
340
540
740
940
4000
3000
2000
1000
0
550
570
590
610
630
650
670
690
200
160
120
80
40
0
Repeat primed peak profile
29/336 CGG
Heterozygous
Full length
336 CGG product
30/30 CGG
Homozygous

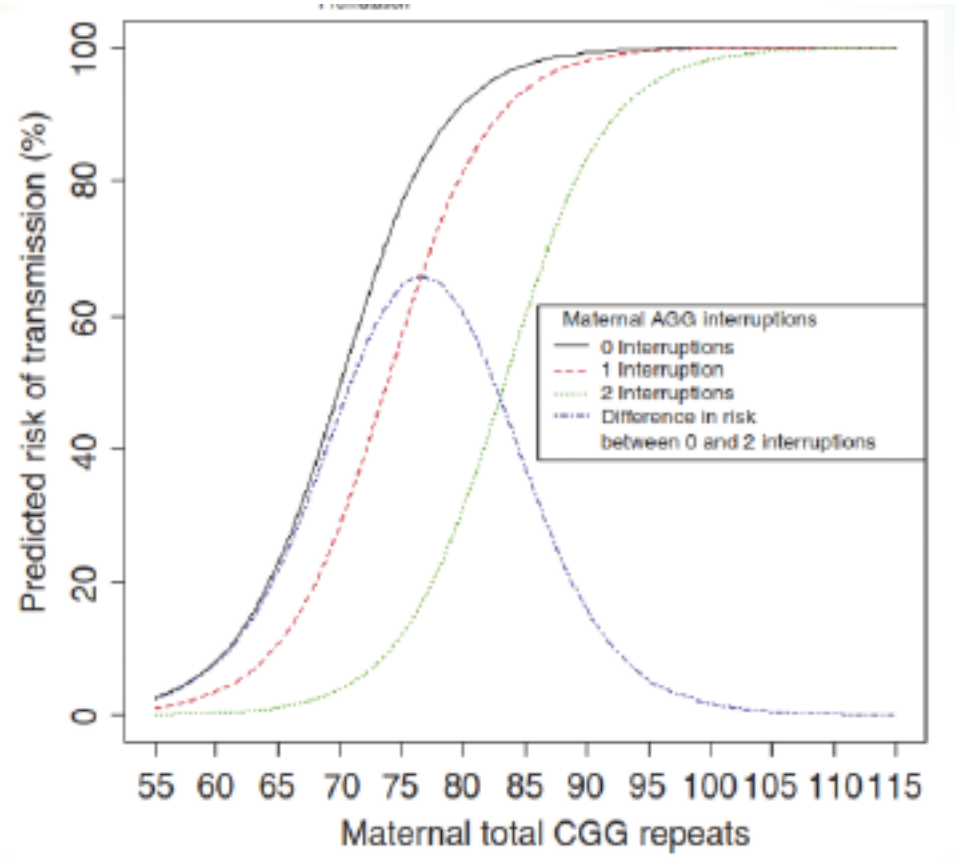
Detection of AGG interruptions







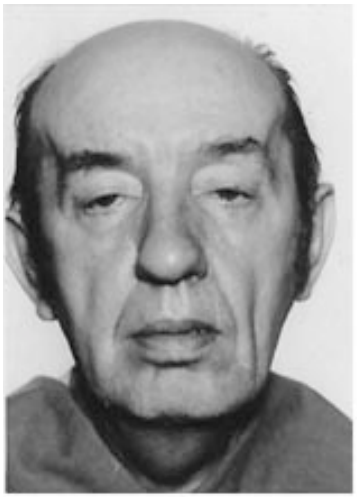
CGG interruptions within the maternal FMR1 gene reduce the risk of offspring with fragile X syndrome



Risk of expansion to full mutation for 75 CGG repeats:

- 0 AGG = 77%
- 2 AGG = 12%

Anticipation phenotype in three-generation families with **dystrophic myotonia (**DM1)

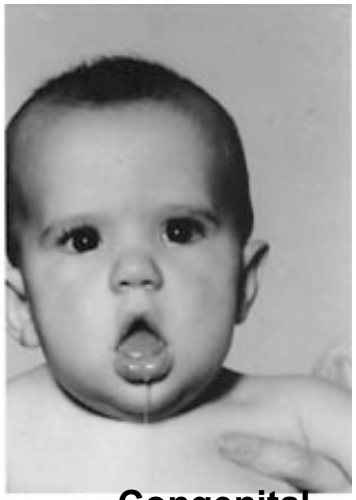


A

Myotonia since age 50

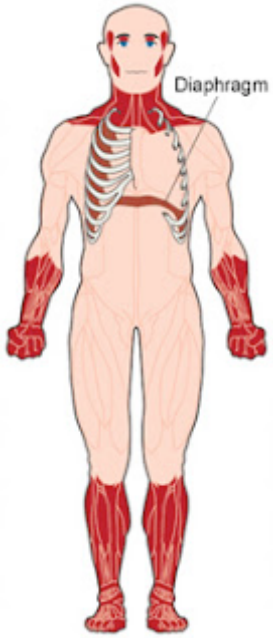
B

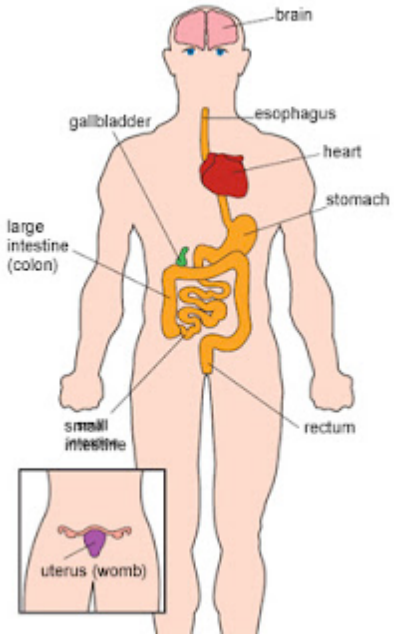
Myotonia since late teens



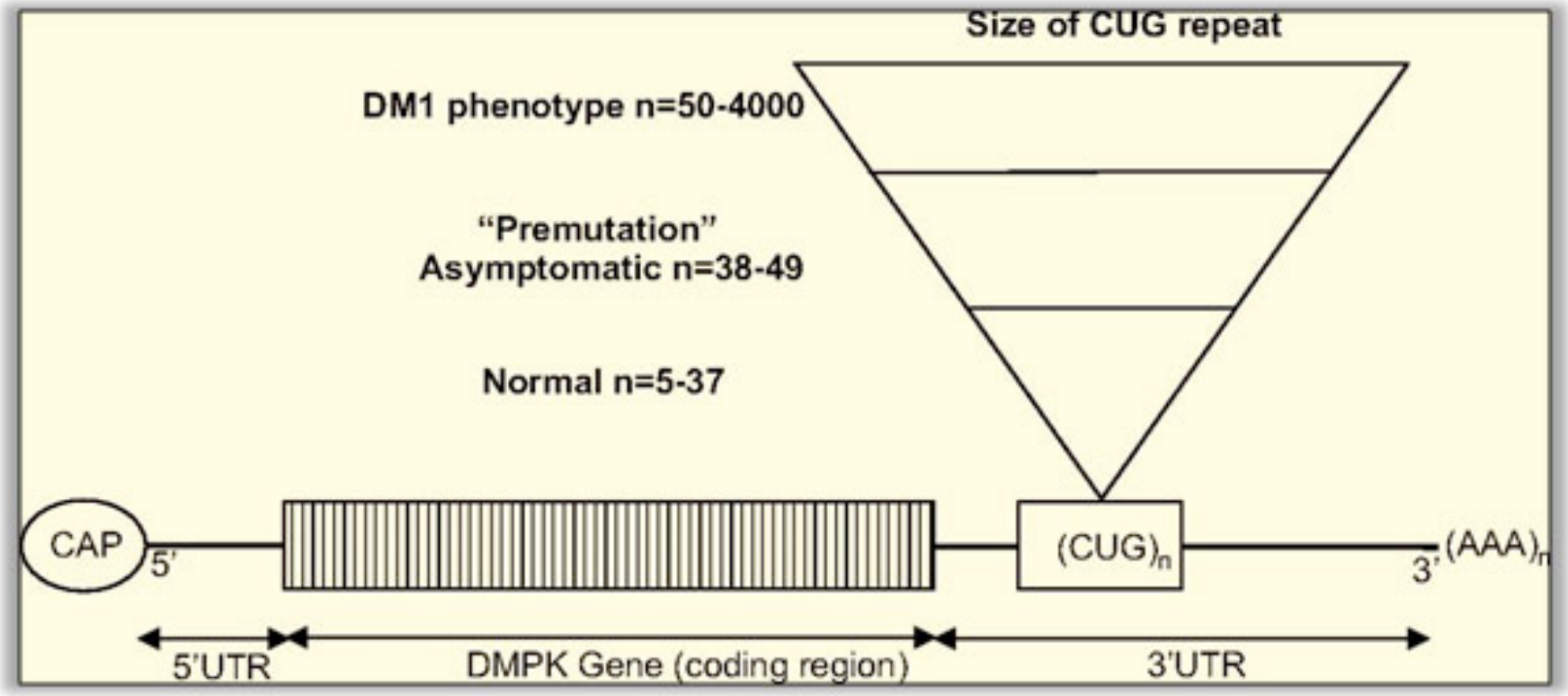
C

Congenital myotonic dystrophy

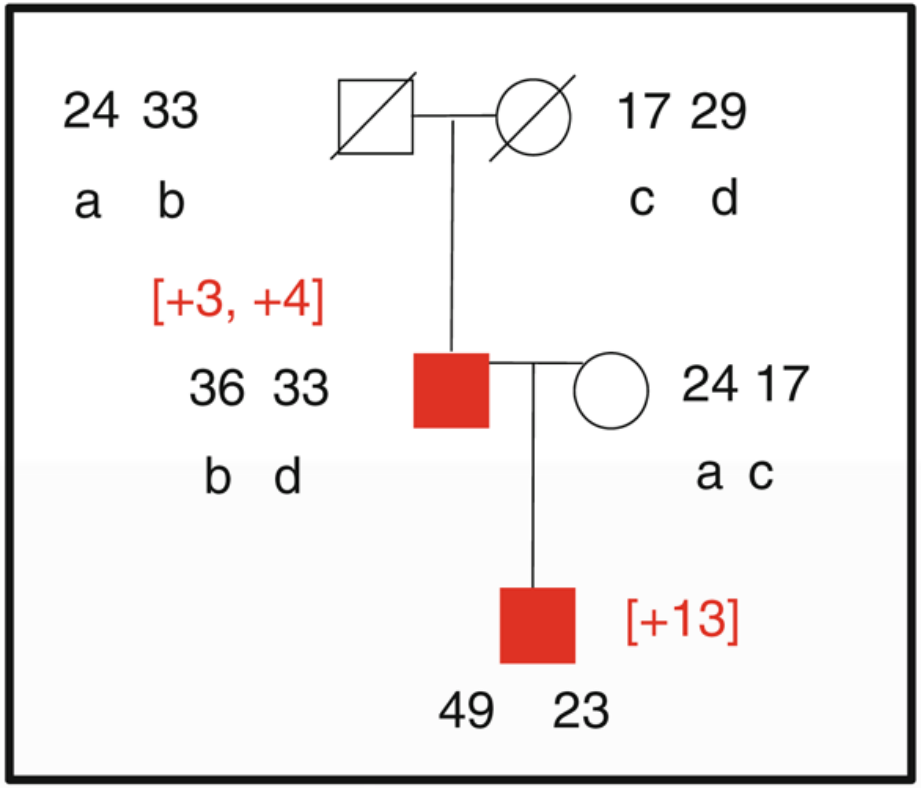


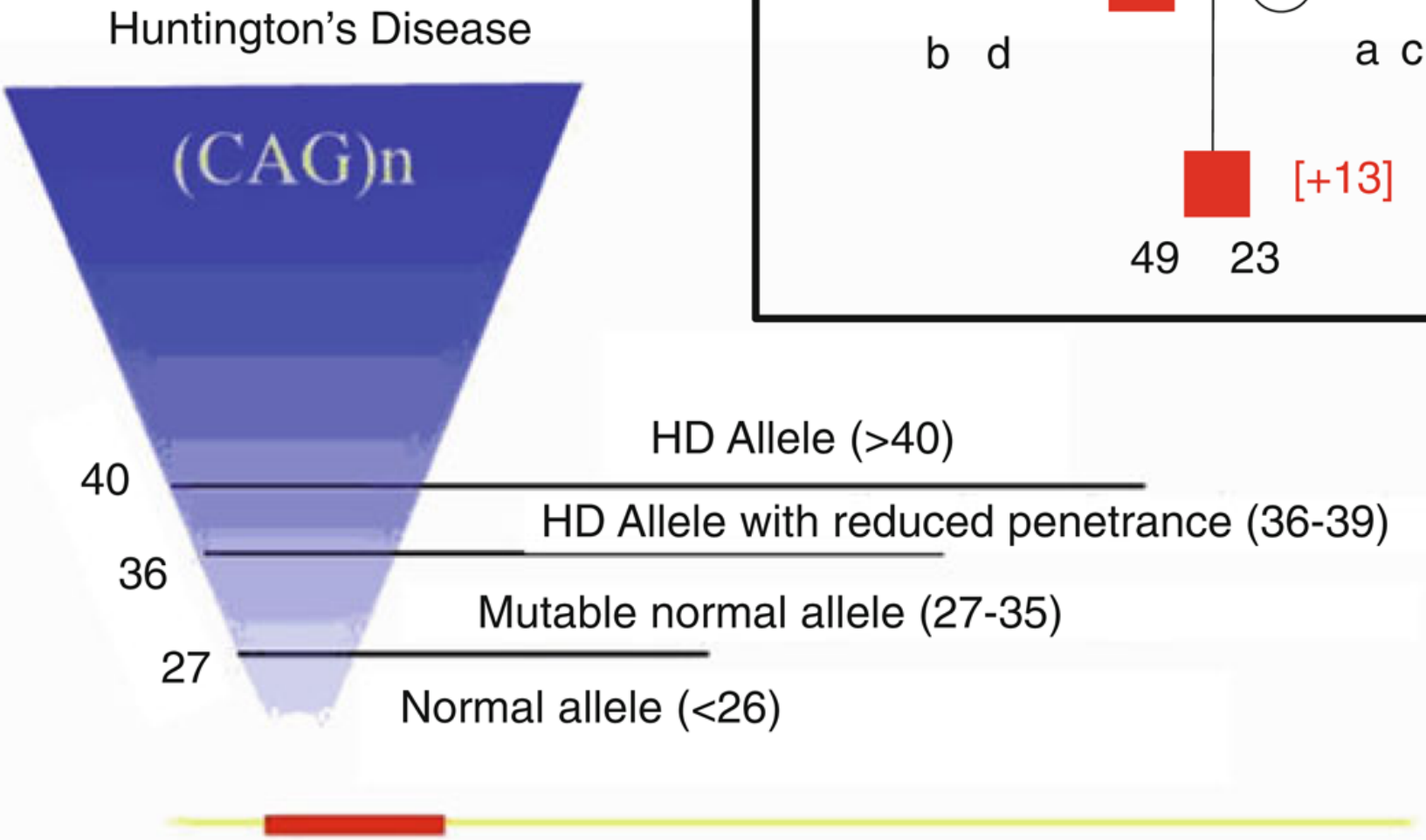


Dystrophic myotonia (DM1): DMPK gene

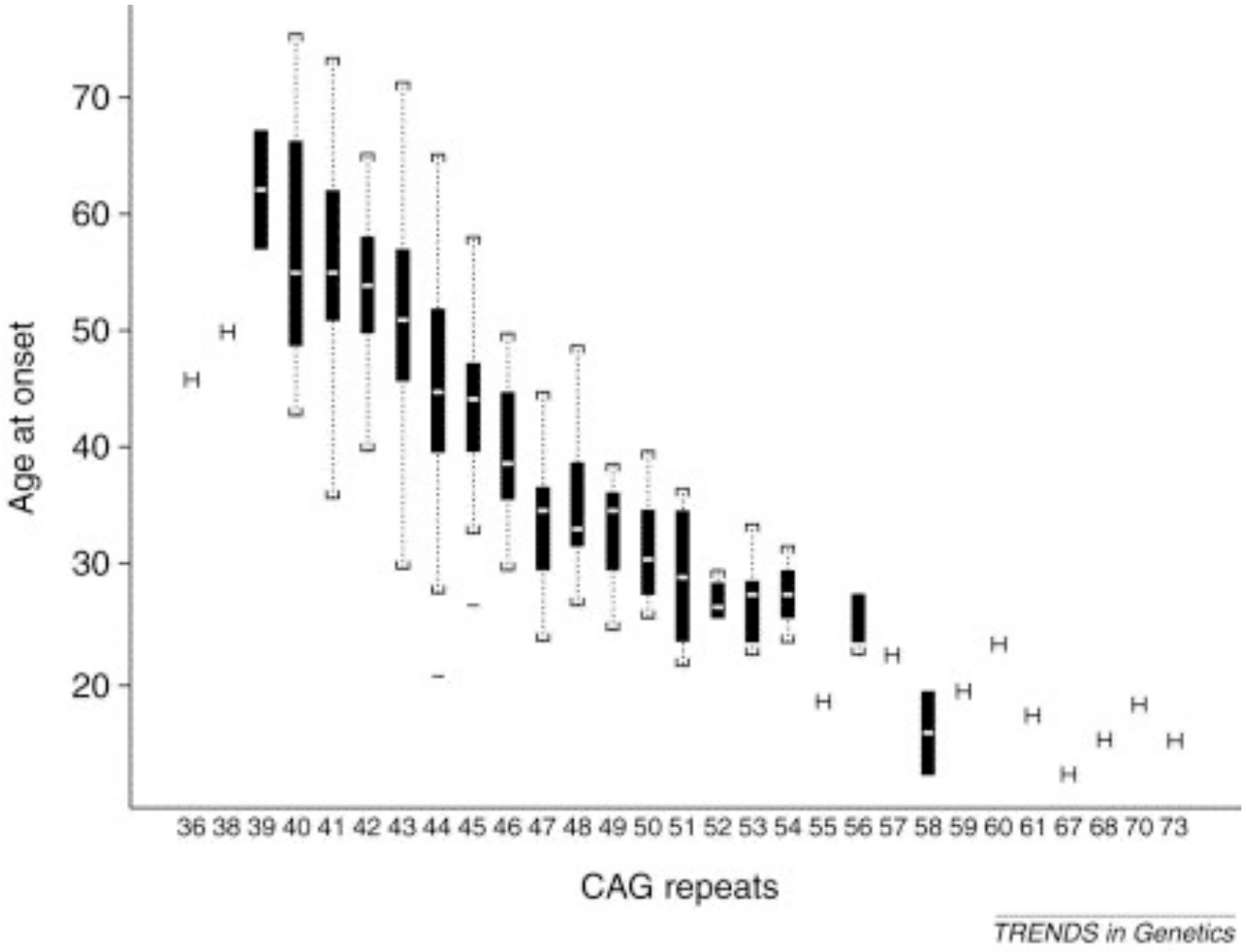


Neurodegenerative disorder producing motor, cognitive and psychiatric symptoms (PolyQ).





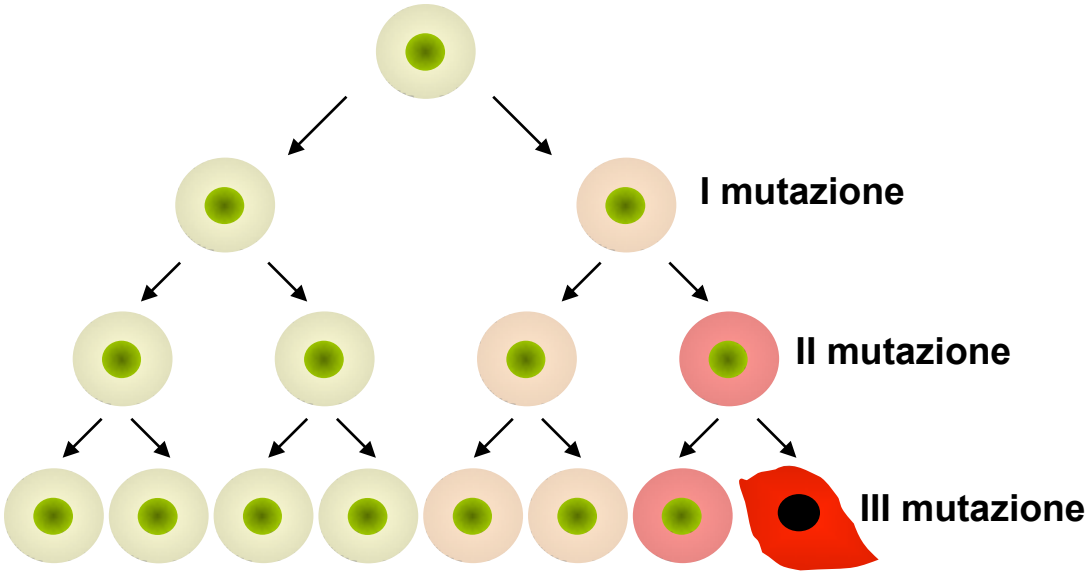
Relationship between CAG repeat number and age of onset of disease

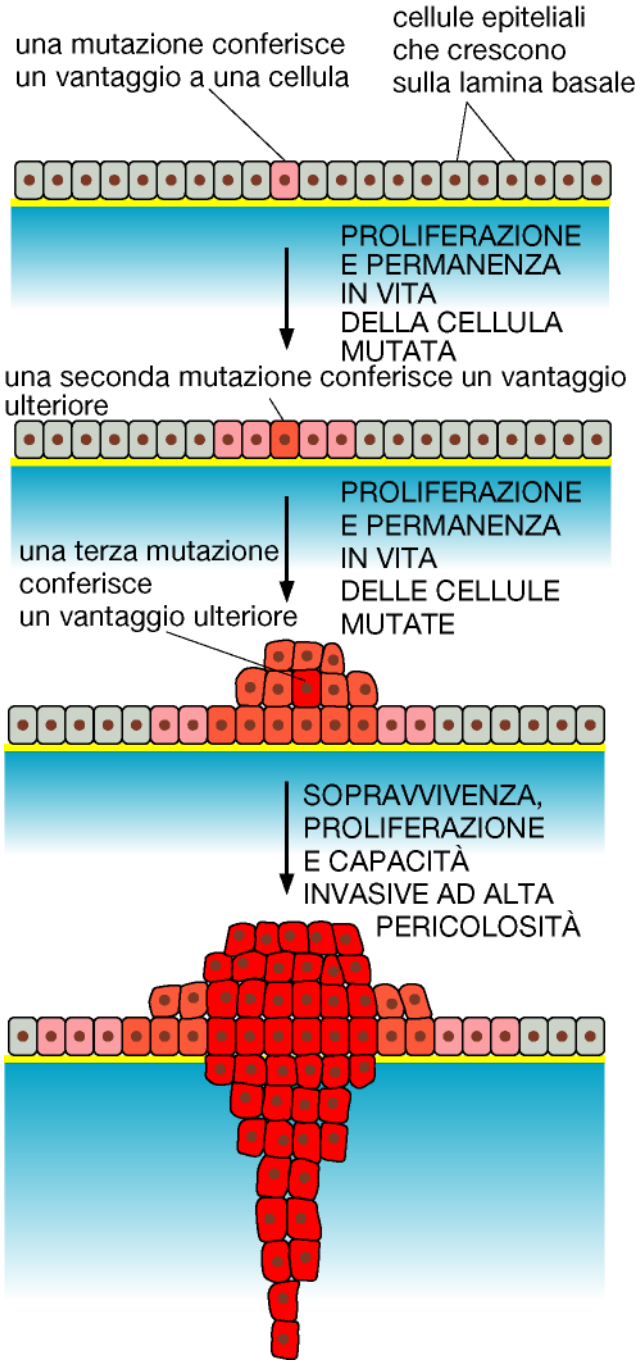


CANCRO: Malattia genetica

- **Geni con diverse funzioni**
  - Controllo del ciclo cellulare
  - Inibizione da contatto
  - Controllo dell'apoptosi
  - Riparazione del DNA
- **Mutazioni con diversi effetti**
  - Acquisizione di funzione ("gain of function")
  - Perdita di funzione ("loss of function")
  - Effetto dominante negativo
  - Anomalie cromosomiche
  - Amplificazione genica

EVOLUZIONE CLONALE:
Accumulo di **mutazioni multiple sequenziali**
di una singola cellula e della sua progenie





How cancer varies over space and time: strategies

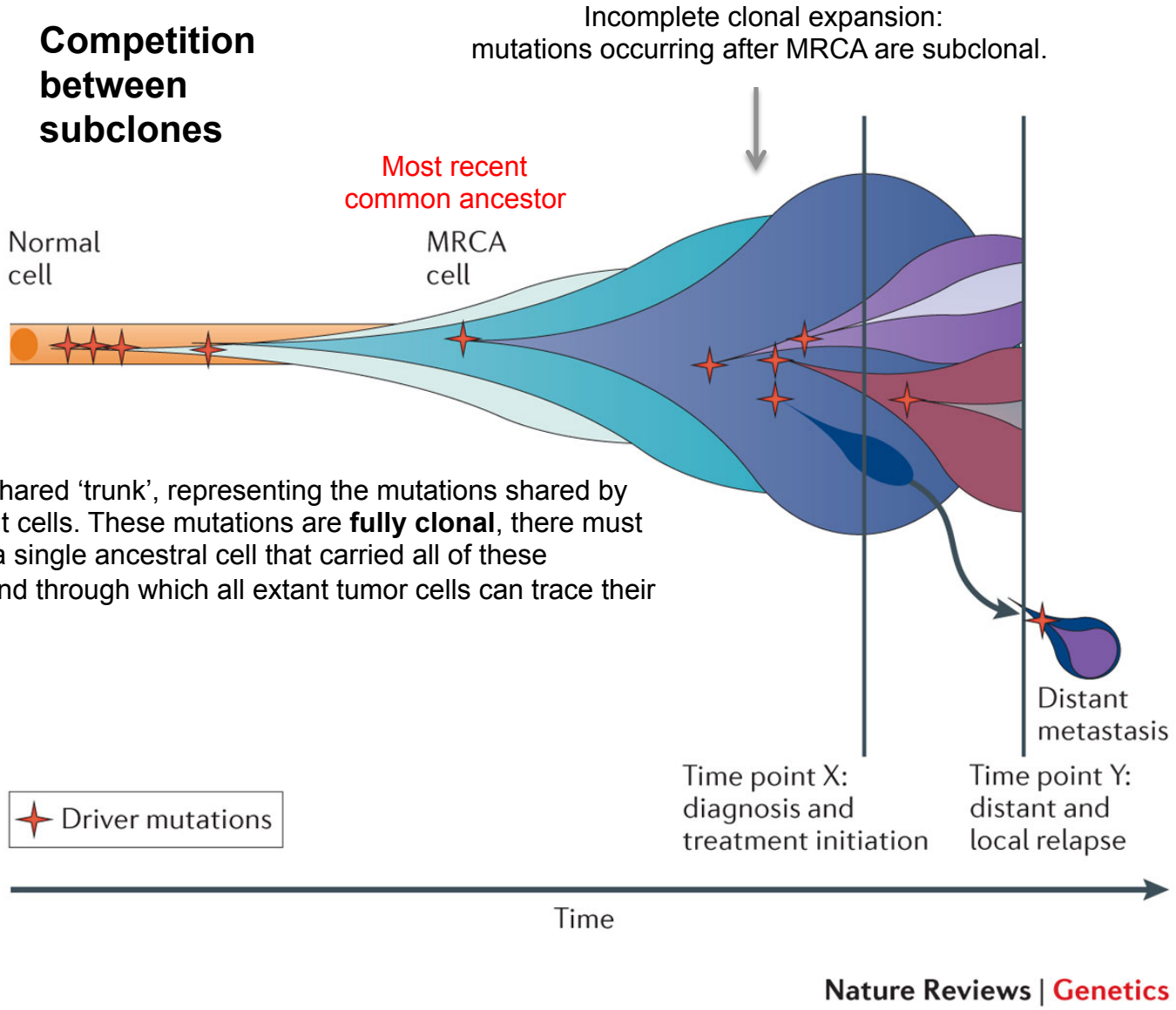
Tumor sampling for sequencing

- Single-cell
- Biopsy specimen (mixed population of cells)

Geographical sampling: multiple samples from an individual cancer obtained at a single point of time

Longitudinal sampling: comparison of samples at different different time points, such as diagnosis, relapse, metastasis

Competition between subclones



There is a shared 'trunk', representing the mutations shared by all malignant cells. These mutations are **fully clonal**, there must have been a single ancestral cell that carried all of these mutations and through which all extant tumor cells can trace their lineage.

Yates and Campbell, 2012

Geni di suscettibilità al Cancro

1. Oncogeni (proto-oncogeni)
2. Geni oncosoppressori
3. Geni di risposta al danno al DNA

ONCOGENI

Stimolano la divisione cellulare

ATTIVAZIONE di oncogeni: "gain of function"
(eccessivamente o non propriamente attivi)

Effetto DOMINANTE: Mutazioni attivanti

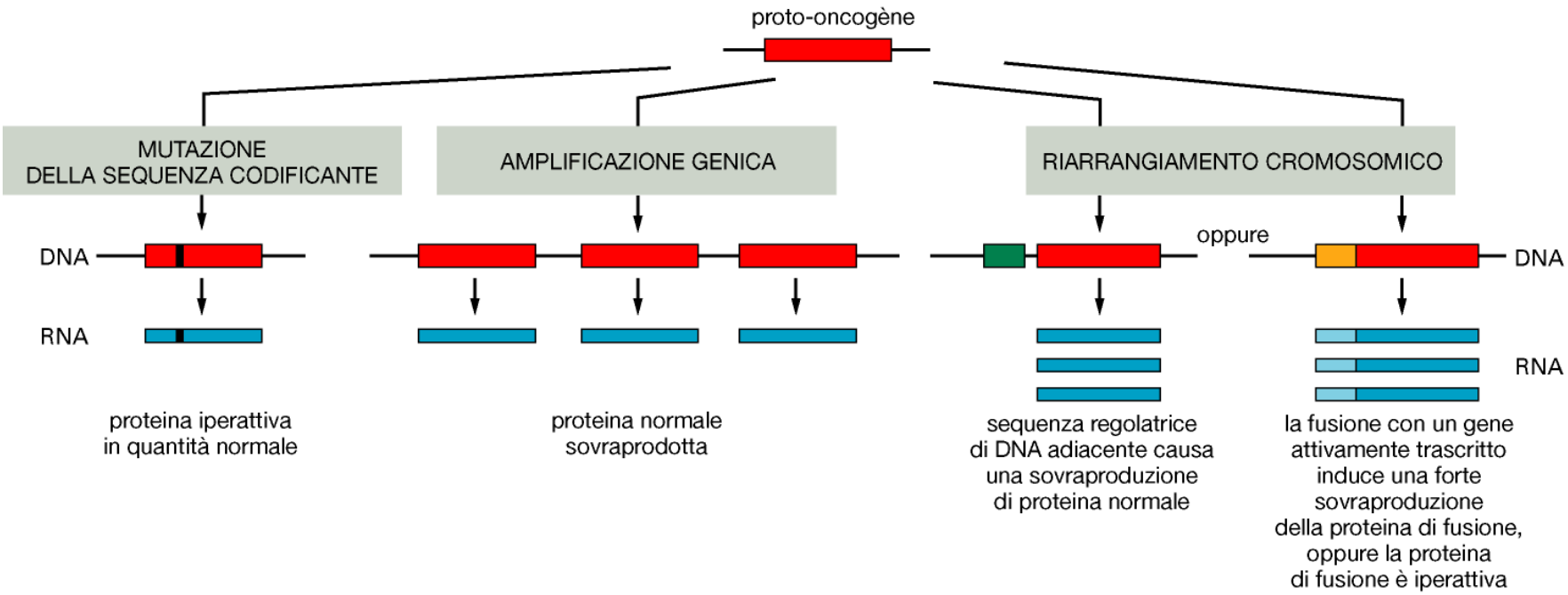
Tumori ereditari (molto rari)

Tumori sporadici (più frequente)

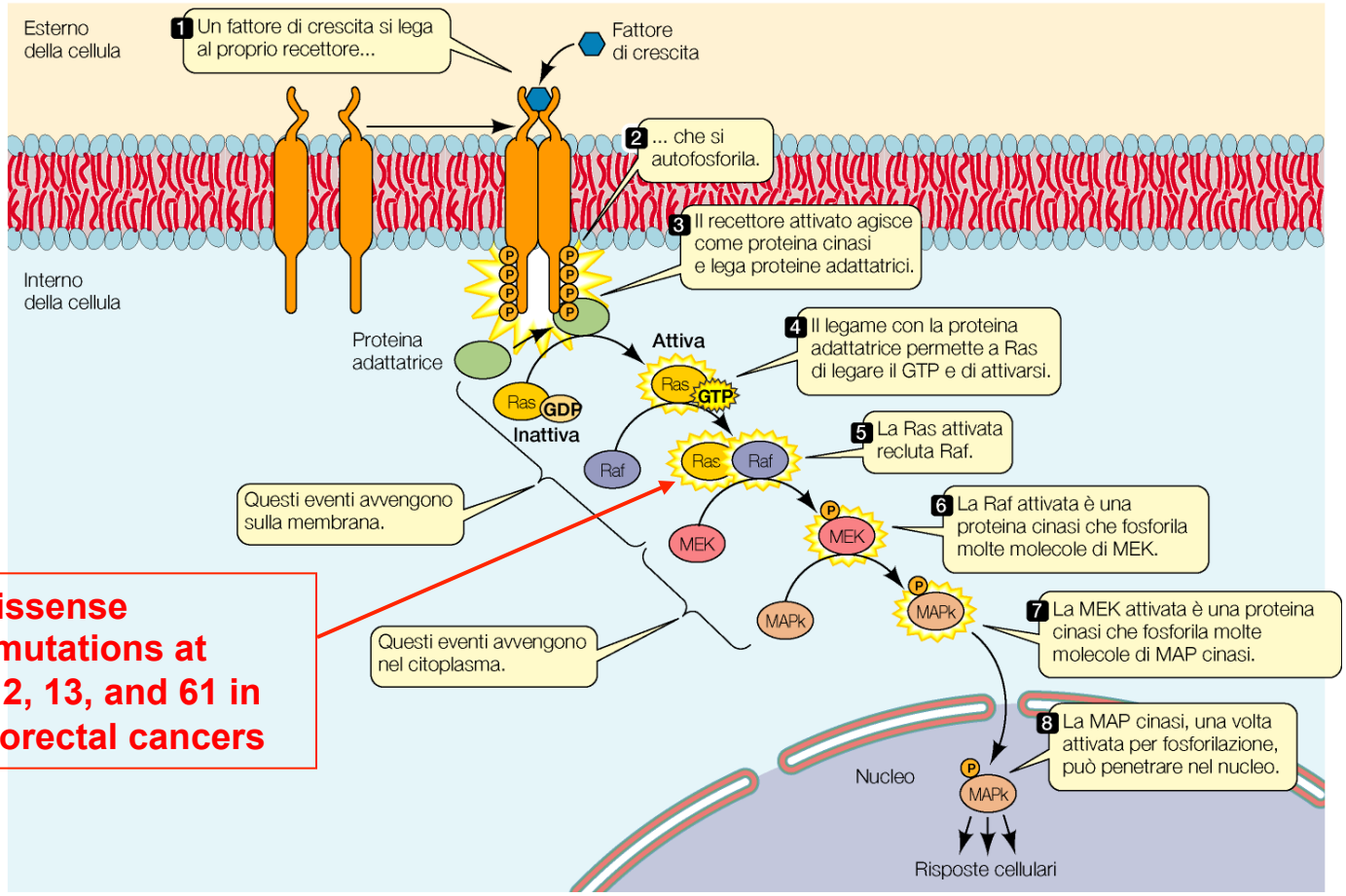
Activaction of oncogenes in hereditary syndromes

Syndrome	Hereditary papillary renal carcinoma (HPCR)
Inheritance	Autosomal dominant
Primary tumor	Renal-cell cancer
Associated cancers and other traits	No
Gene	MET (Activacted oncogene)
Chromosomal localization	7q31
Proposed function of gene product	Transmenbrane tyrosine kinase receptor for hepatocyte growth factor
Clonality	Trisomy (duplication of chromosome 7 bearing the activacted MET gene)

Meccanismi di attivazione di proto-oncogeni
Difetti qualitativi o quantitativi



1) Mutazione nella sequenza codificante attivazione costitutiva di Ras mediante mutazioni missense



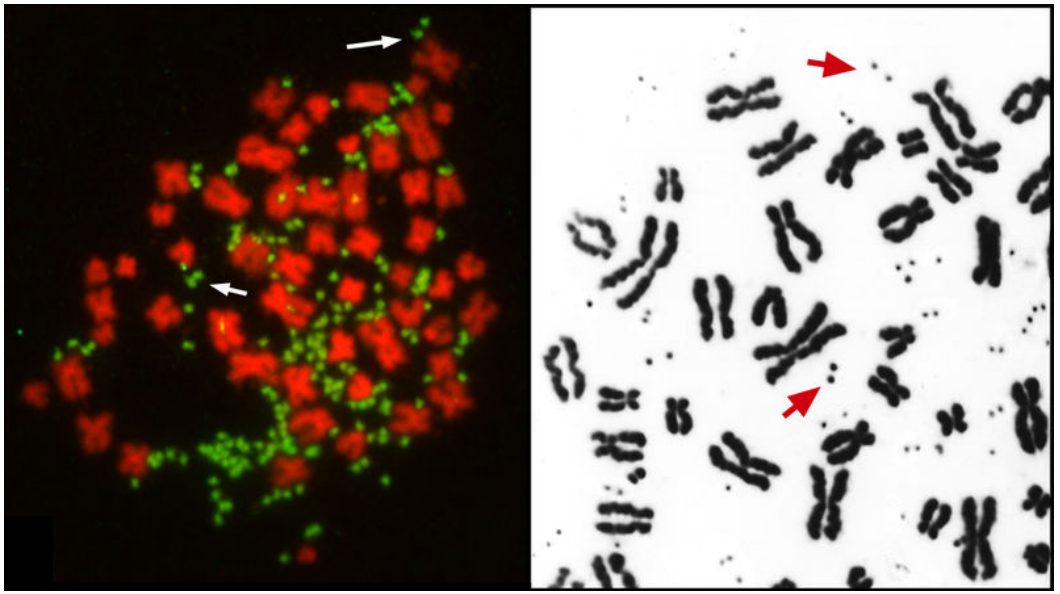
Somatic missense activating mutations at positions 12, 13, and 61 in 30-40% colorectal cancers

2) Amplificazione genica

Amplificazione di un frammento di DNA centinaia di volte che, molto probabilmente, contiene geni che favoriscono la progressione del tumore. Si osserva in numerosi tumori.

- **Homogeneously Staining Regions (HSRs)**
  Segmento di DNA amplificato molte volte all'interno di un cromosoma normale

- **Double minutes**
  Piccoli corpi di DNA extracromosomico circolare che si replicano nel nucleo prima della divisione cellulare



3) Riarrangiamenti cromosomici: traslocazioni

Neoplasm	Chromosomal translocation	% of cases	Proto-Oncogene affected
Burkitt lymphoma	t(8;14)(q24;q32)	0.80	MYC
	t(8;22)(q24;q11)	0.15	
	t(2;8)(q11;q24)	0.05	
Chronic myelogenous leukemia	t(9;22)(q34;q11)	0.90-0.95	ABL-BCR
Acute lymphocytic leukemia	t(9;22)(q34;q11)	0.10-0.15	BCR-ABL
Acute Lymphoblastic leukemia	t(1;19)(q23;p13)		PRL homeobox gene
Acute promyelocytic leukemia	t(15;17)(q22;q11)		PML-RARA
Chronic lymphocytic leukemia	t(11;14)(q13;q32)	0.10-0.30	BCL-1
Follicular lymphoma	t(14;18)(q32;q21)		BCL-2

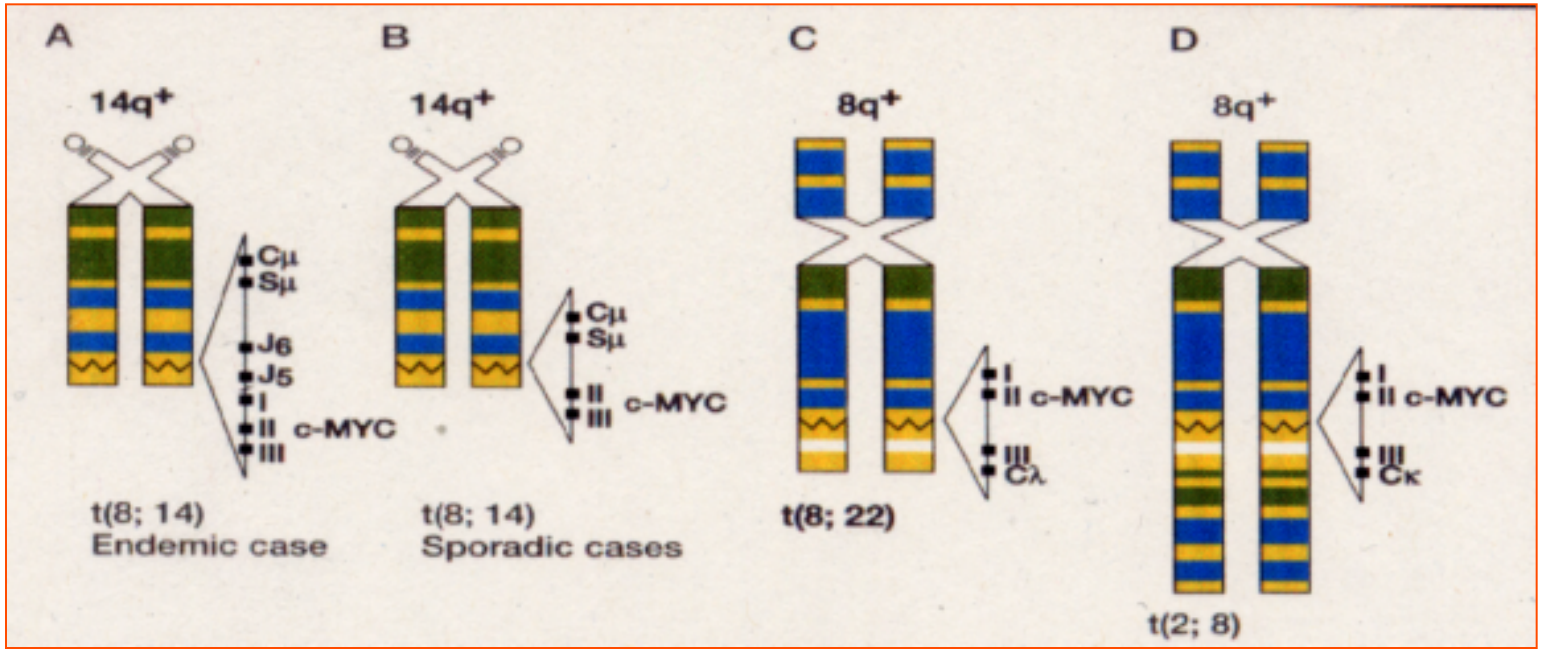
Linfoma di Burkitt

esempio di traslocazione in una regione attiva trascrizionalmente

IGH
14q32

IGL
22q11

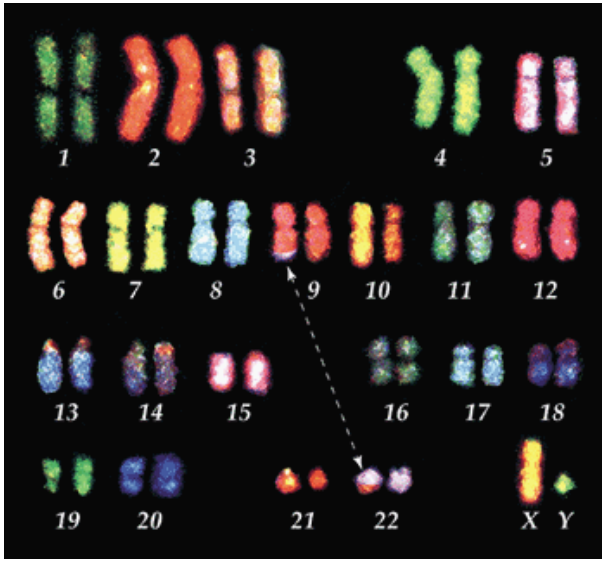
IGK
2p12

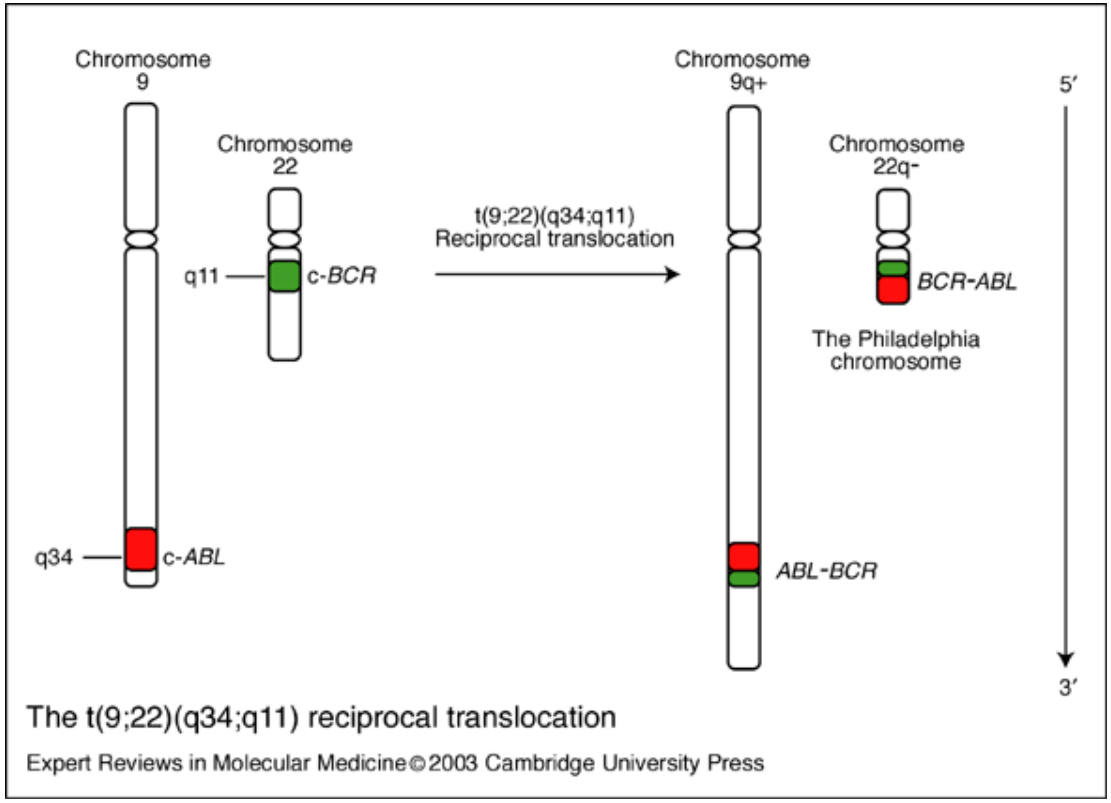


MYC traslocato in loci Ig che producono anticorpi e che sono attivamente espressi in cellule B

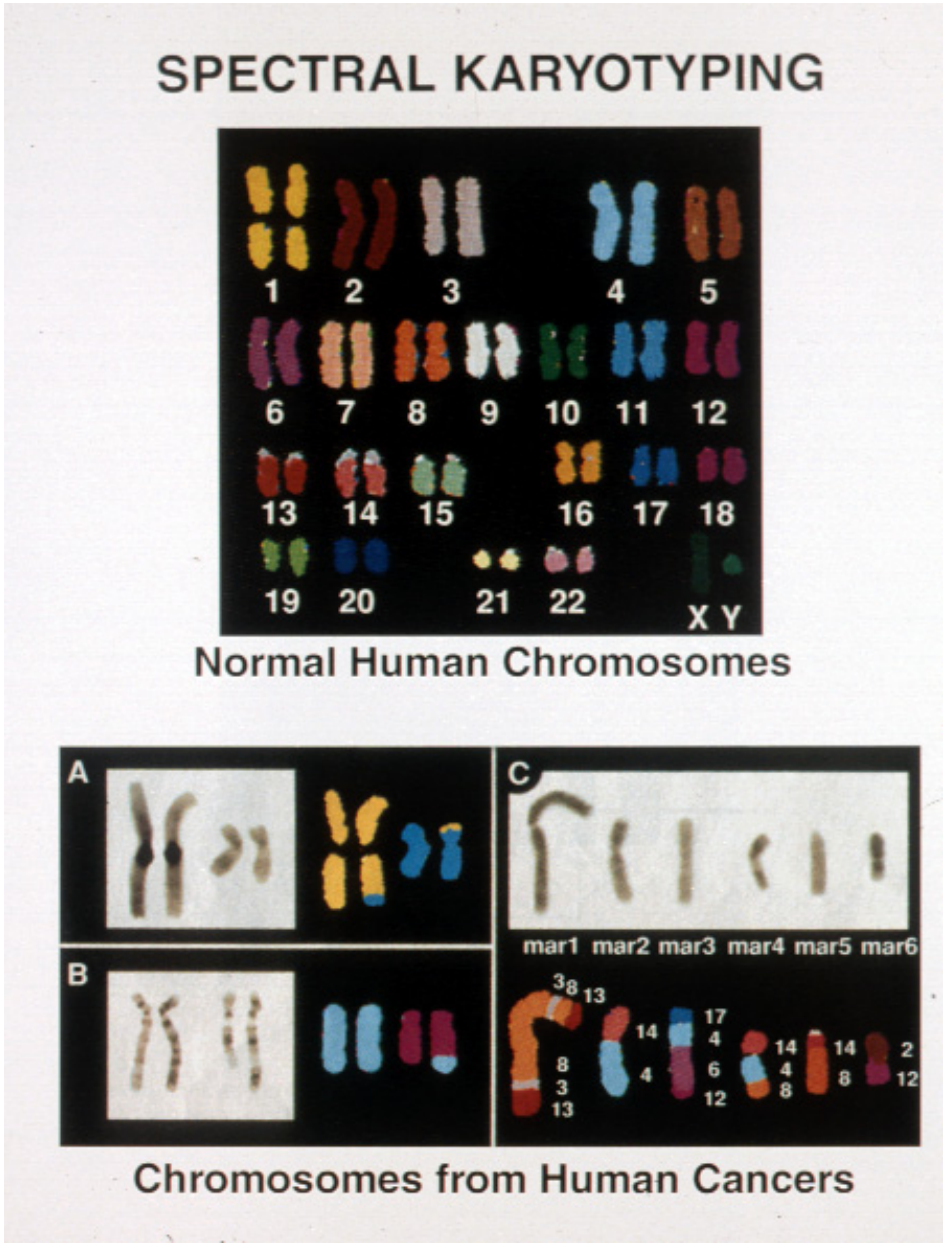
LEUCEMIA MIELOIDE CRONICA (CML)

Esempio di traslocazione cromosomica che genera un nuovo gene (gain of function)

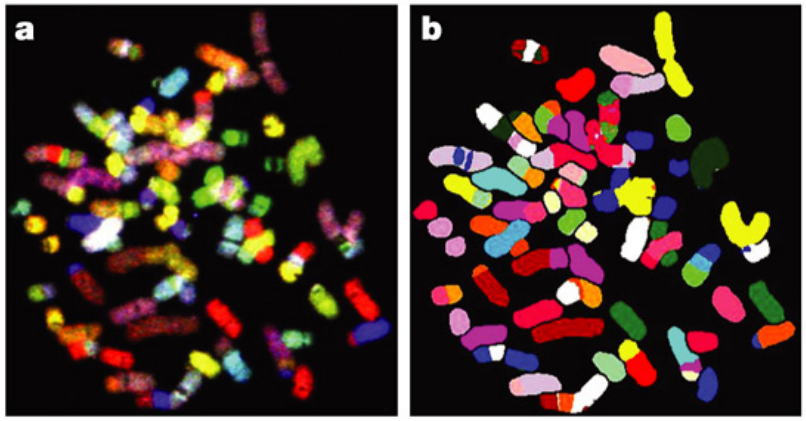




BRC-ABL proteina di fusione con attività chinasica costitutiva



Spectral karyotyping: utilizzo di sonde marcate con coloranti diversi per ciascun cromosoma



Nature Reviews | Cancer

Riarrangaimenti cromosomici cancro-specifici

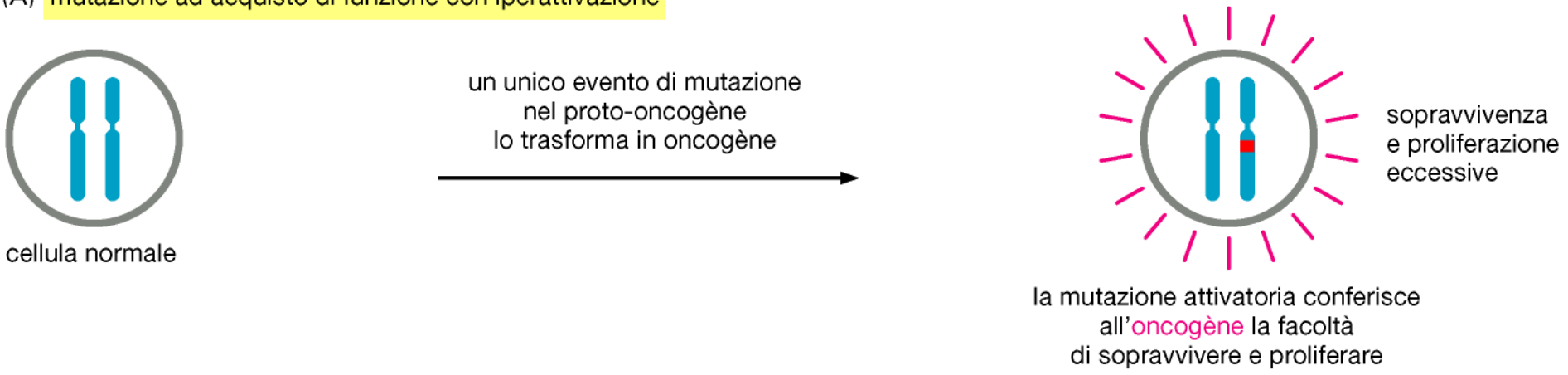
Cancer genome anatomy project (CGAP)
>40000 alterazioni cromosomiche

Mitelman Database of Chromosome Aberrations in Cancer

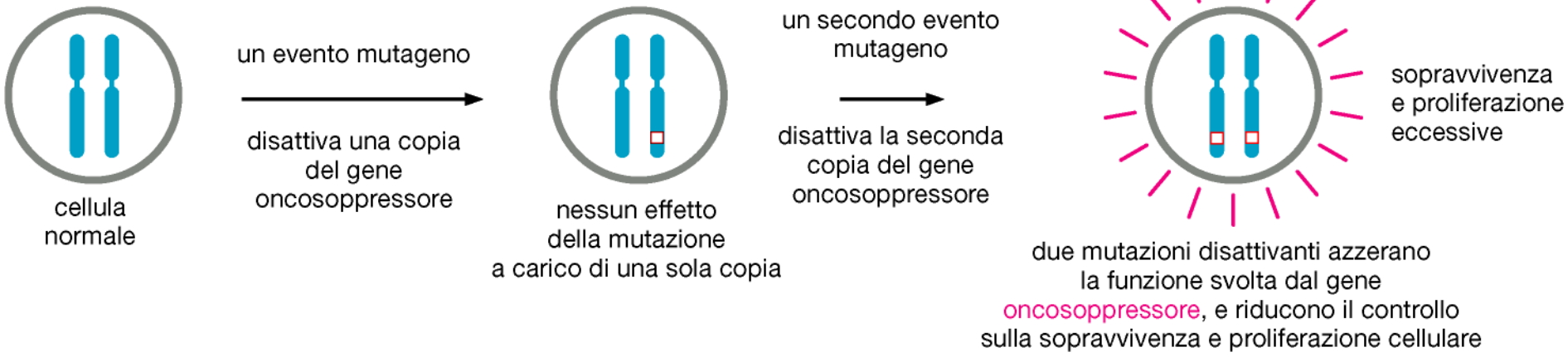
http://cgap.nci.nih.gov/Chromosomes/Mitelman

ONCOGENI

(A) mutazione ad acquisto di funzione con iperattivazione



(B) mutazione a perdita di funzione con disattivazione



GENI ONCOSOPPRESSORI

Geni oncosoppressori

Controllano la proliferazione cellulare

INATTIVAZIONE di geni oncosoppressori: perdita di funzione

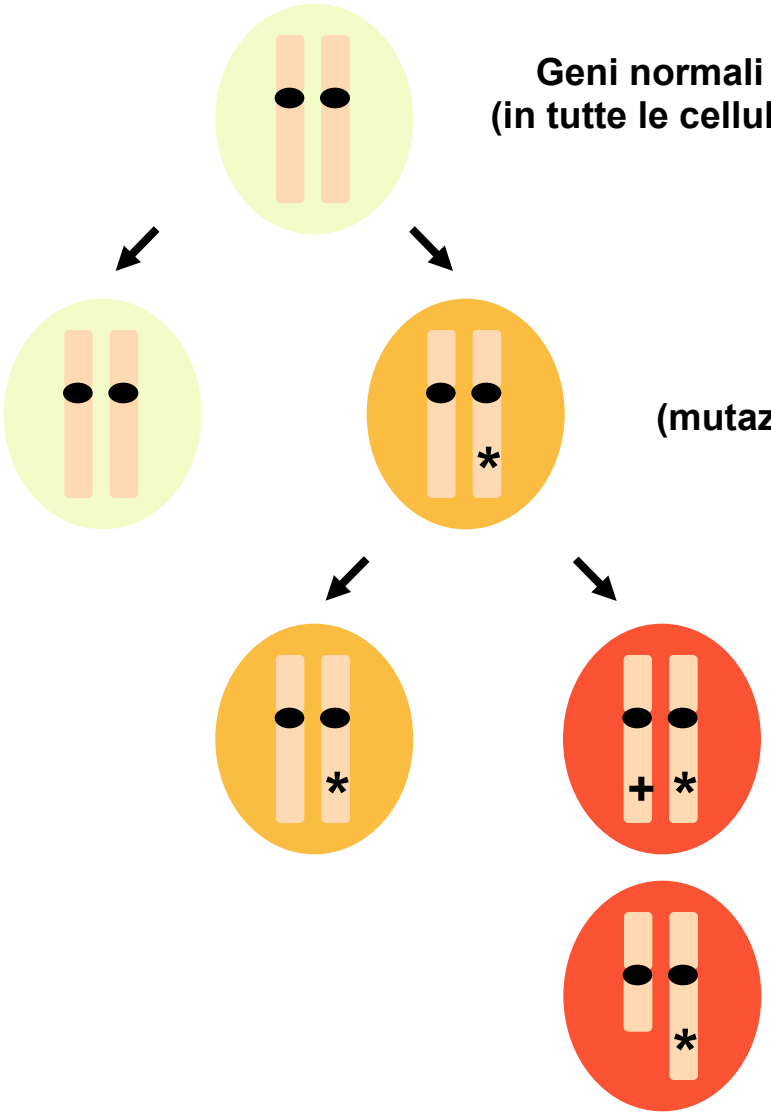
Effetto RECESSIVO: entrambi gli alleli sono inattivati

IPOTESI "TWO-HITS"
Tumori ereditari e sporadici

Tumori sporadici
2 mutazioni somatiche

Tumori ereditari
1 mutazione germinale + 1 mutazione somatica

TUMORE SPORADICO: 2 Mutazioni somatiche



Geni normali
(in tutte le cellule)

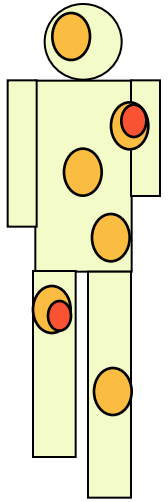
Perdita di un allele
(mutazione somatica in una o più cellule)

Perdita del secondo allele

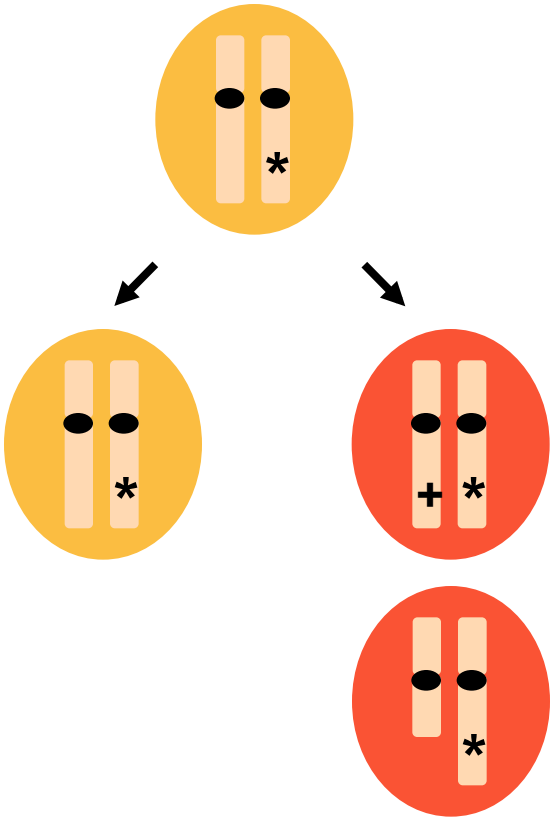
Mutazione

Inattivazione/metilazione

LOH



TUMORE EREDITARIO:
Mutazione germinale + mutazione somatica



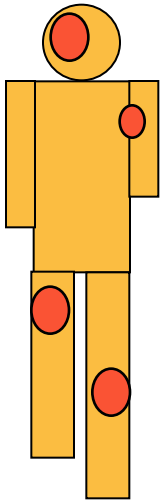
Perdita di un allele
(mutazione ereditata da un genitore in tutte le cellule)

Perdita del secondo allele

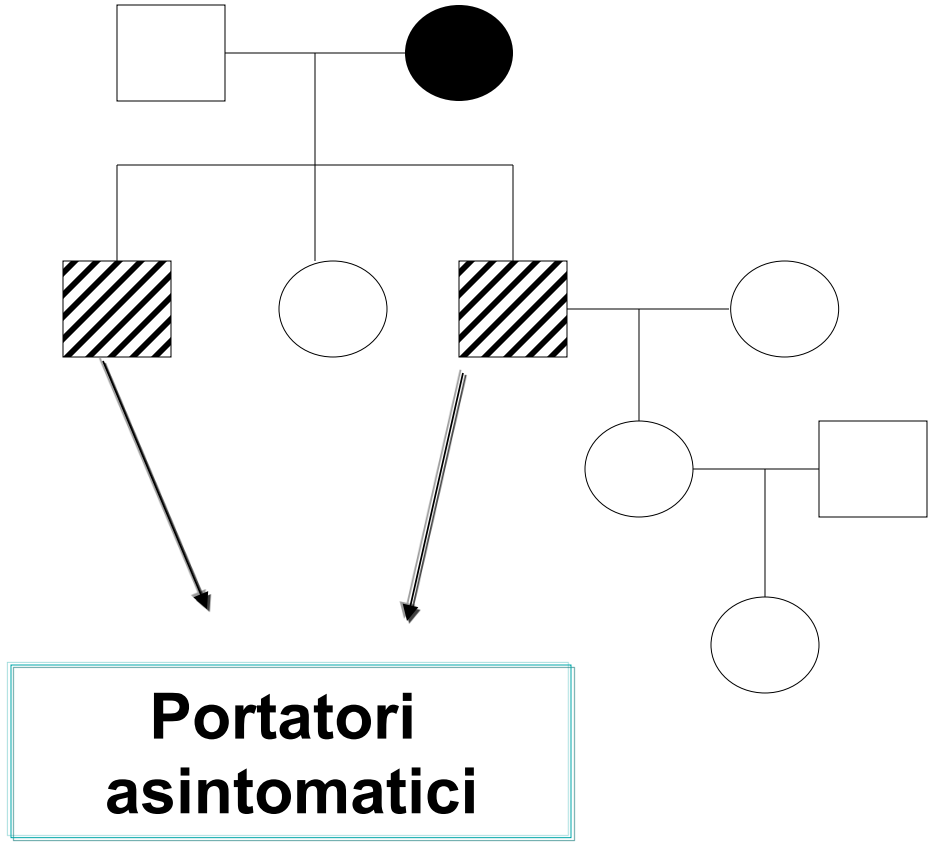
Mutazione

Inattivazione/metilazione

LOH



Ciò che viene ereditato è la PREDISPOSIZIONE



Tumori ereditari (5%): potenziali indicatori

- **Casi multipli nella famiglia in più generazioni (parenti di primo grado)**
- **Specifici tipi di tumore nello stesso individuo o in membri diversi della famiglia**
- **Riconoscimento di una sindrome con predisposizione a tumori**
- Esordio ad un' età più giovane rispetto ai tumori sporadici
- **Diagnosi di tumore con nota componente ereditaria (retinoblastoma, tumore midollare della tiroide, feocromocitoma)**
- **Mutazione identificata in gene-tumore in un membro della famiglia**

Consulenza genetica

Identificare famiglie e individui a rischio
Test molecolari di screening di mutazioni
Prevenire tumore e fornire terapie adeguate
Diagnosi prenatale

Geni Oncosoppressori implicati in Tumori Ereditari

Gene	Malattia
APC	FAP (Familial Adenomatous Polyposis)
VHL	Syndrome di Von Hippel-Lindau
WT1	Tumore di Wilms Ereditario
RB1	**Retinoblastoma Ereditario**
NF1	Neurofibromatosi 1
NF2	Neurofibromatosi 2
p53	Sindrome di Li Fraumeni
P16/CDK4	Melanoma Ereditario
PTCH	Nevoid Basal Cell carcinoma syndrome
MEN1	MEN (Multiple Endocrine Neoplasia) tipo 1
BRCA1 BRCA2	**Carcinoma Ereditario Mammella/Ovaio**

RETINOBLASTOMA (MIM 180200)

Raro tumore embrionario della retina

Autosomico dominante

1/15-18.000 nati
3% dei tumori in età pediatrica

Insorgenza: nei primi 3 anni, difficilmente dopo i 7 anni

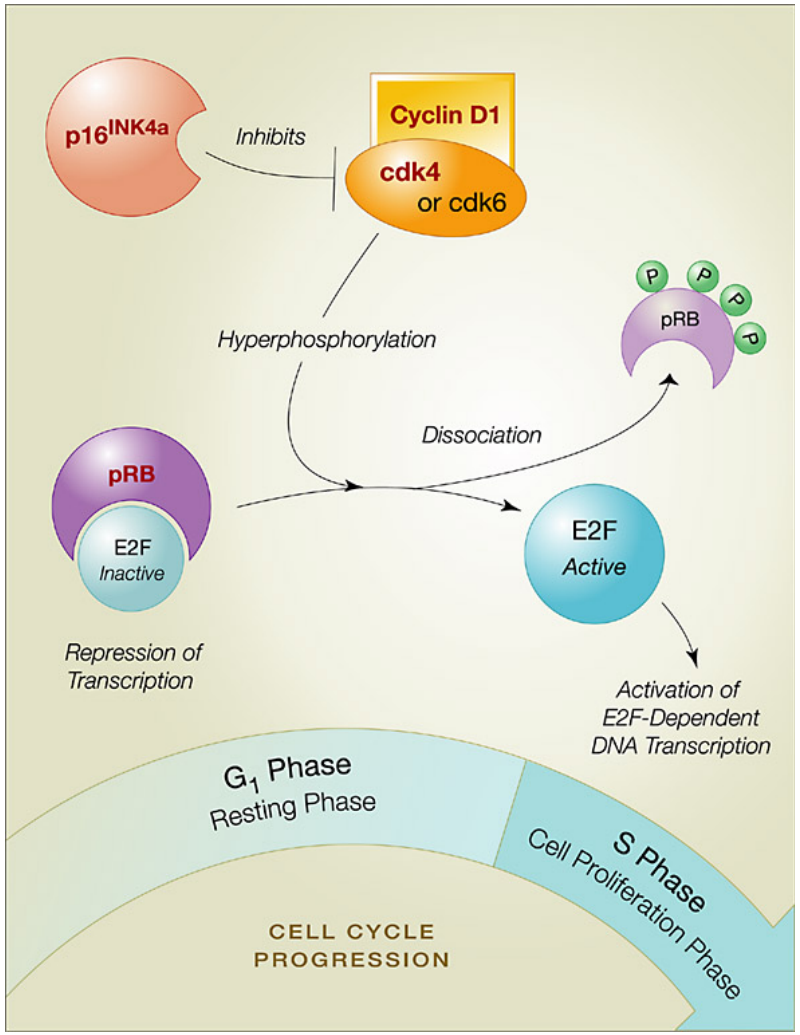
Sporadico/Ereditario

Unilaterale/Bilaterale

Unifocale/Multifocale

Mutazioni in RB1 (oncosoppressore)

PATHWAY RB1: controllo del ciclo cellulare



Perdita del gene RB

↓

Proliferazione incontrollata

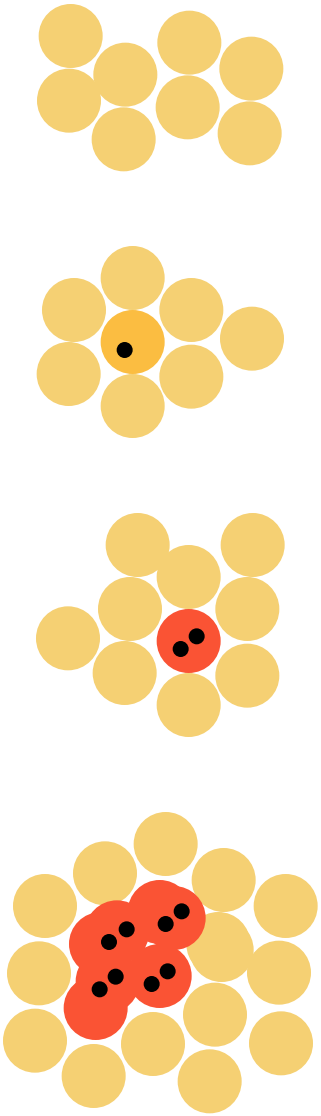
↓

Tumori

Livingston and Shivdasani, JAMA 285:588, 2001

RETINOBLASTOMA SPORADICO

RETINA

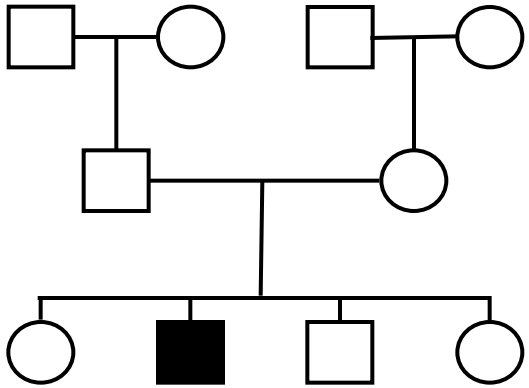


Gene normale

I mutazione somatica

II mutazione somatica

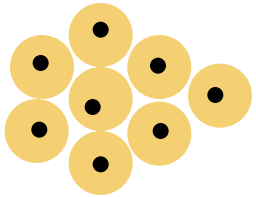
Tumore unilaterale
unifocale
Insorgenza tardiva
(24-30 mesi)



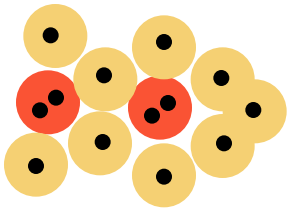
NON rischio 2° tumore
NON rischi da terapia radiante
NON trasmissione ereditaria

RETINOBLASTOMA EREDITARIO

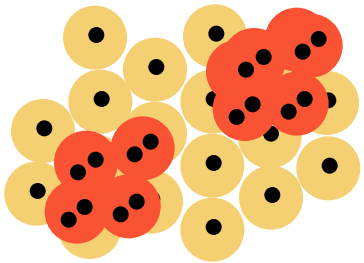
RETINA



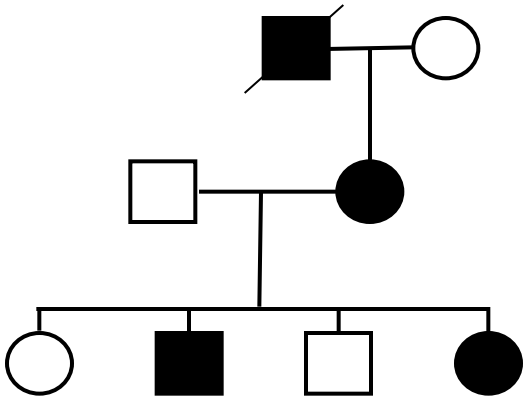
Mutazione germinale
tutte le cellule



Mutazione somatica

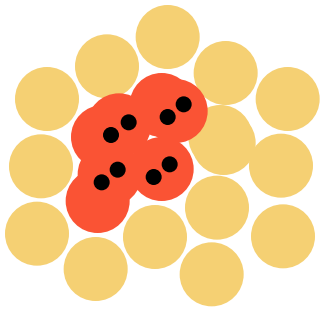


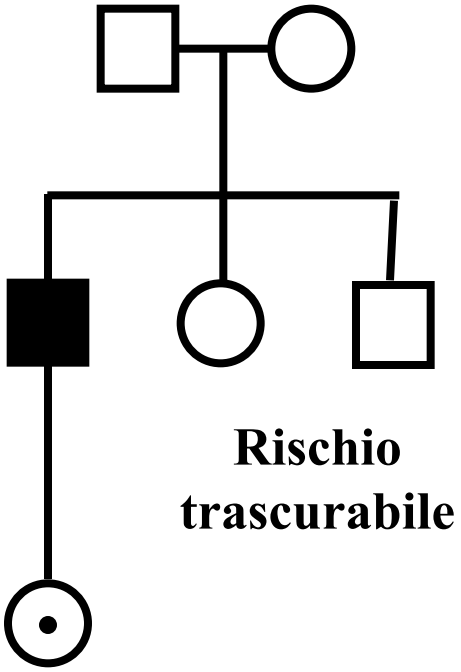
Tumore bilaterale
(Unilaterale > bilaterale)
Multifocale
Insorgenza precoce
(<12 mesi, anche in utero)



Rischio 2° tumore (RR=400)
(osteosarcoma, fibrosarcoma, melanoma)
Rischi da terapia radiante
Trasmissione ereditaria
Autosomica dominante

RETINOBLASTOMA SPORADICO
60%



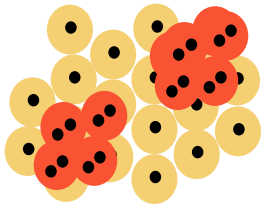


Rischio trascurabile

1/100

RETINOBLASTOMA EREDITARIO (40%)

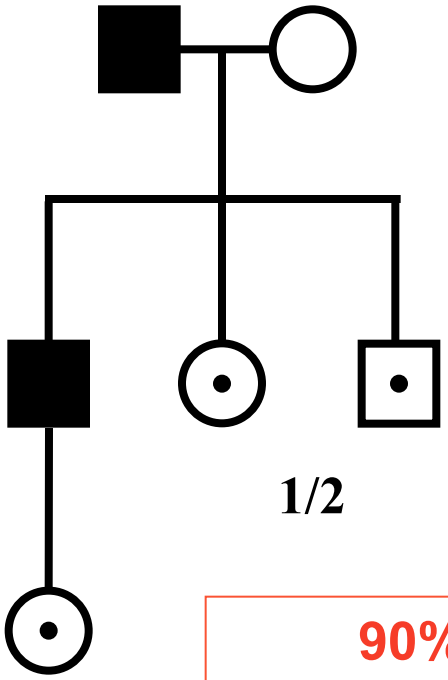
Mutazione germinale nei genitori (10-15%)



Mutazione germinale assente nei genitori (85-90%)

"de novo" (90-95%)

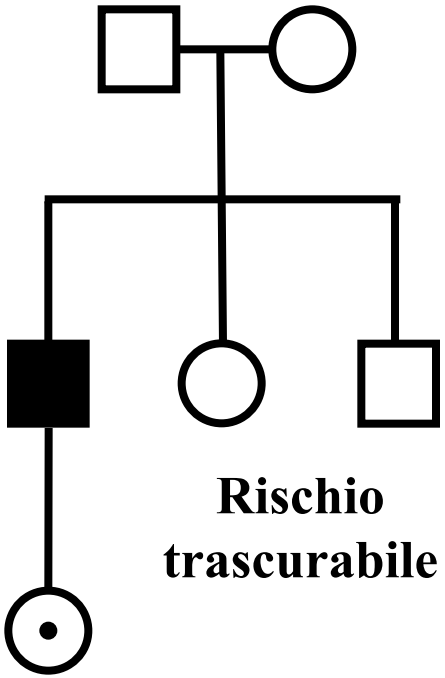
Mosaicismo germinale (5-10%)



1/2

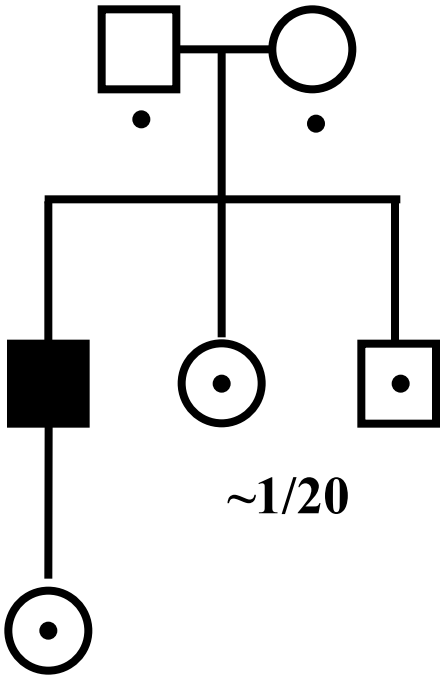
1/2

90% rischio tumore



Rischio trascurabile

1/2



~1/20

1/2

Mutazioni nel gene RB1

Anomalie cromosomiche (15%)
(delezioni, inserzioni, duplicazioni, traslocazioni)

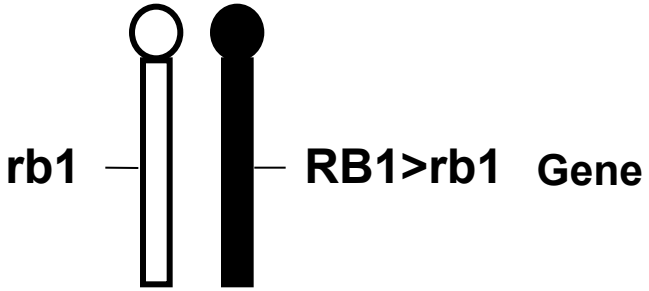
Piccole delezioni, inserzioni, mutazioni complesse (15%)

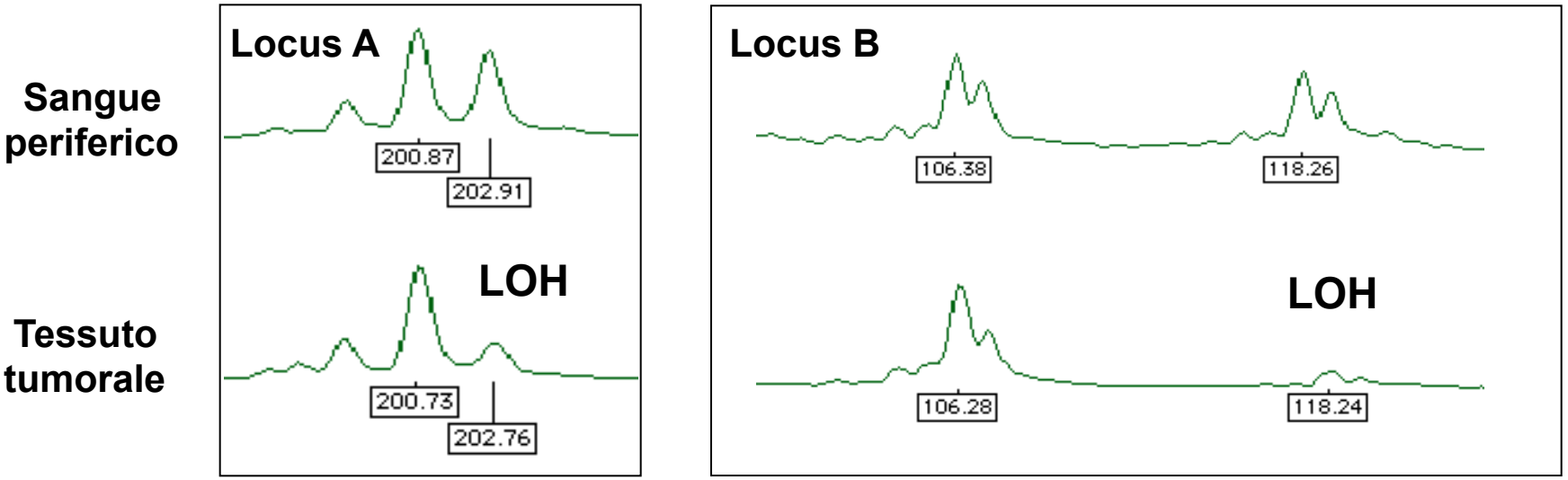
Sostituzioni nucleotidiche (70%)
(mutazioni missense, nonsense, frameshift, promotore)

RB1

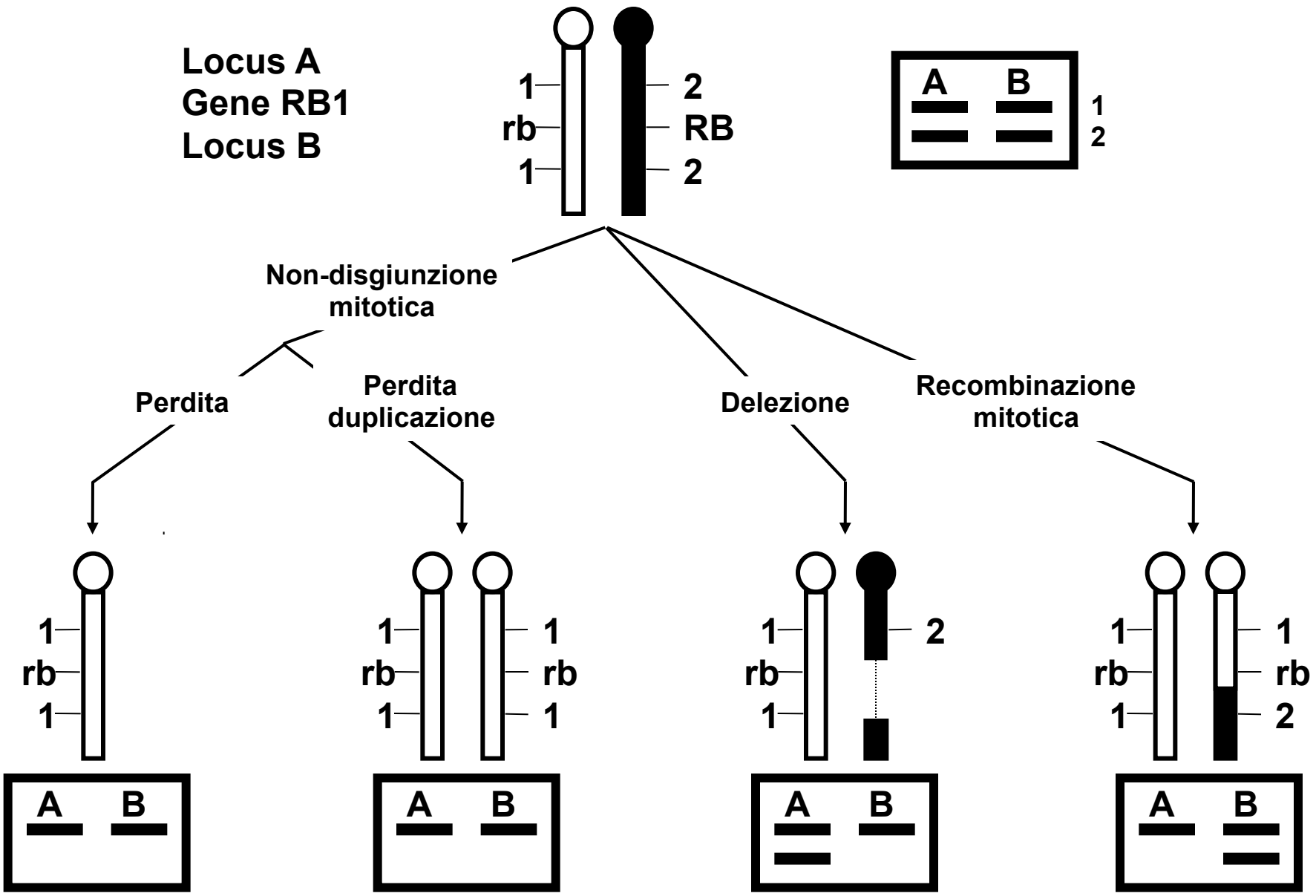
13q14

LOH: loss of heterozygosity

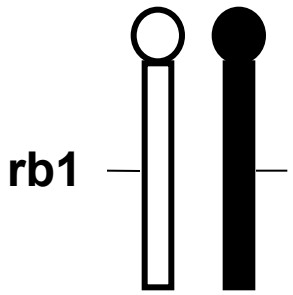




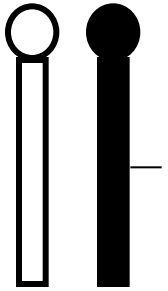
Meccanismi implicati nella LOH



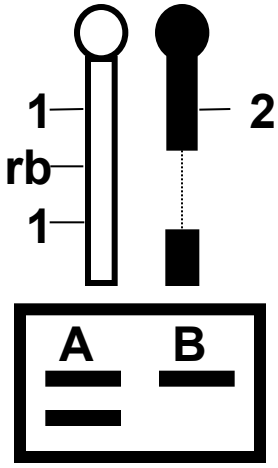
Meccanismi perdita di funzione implicati nel secondo “hit”



Mutazione
Somatica
in RB wt

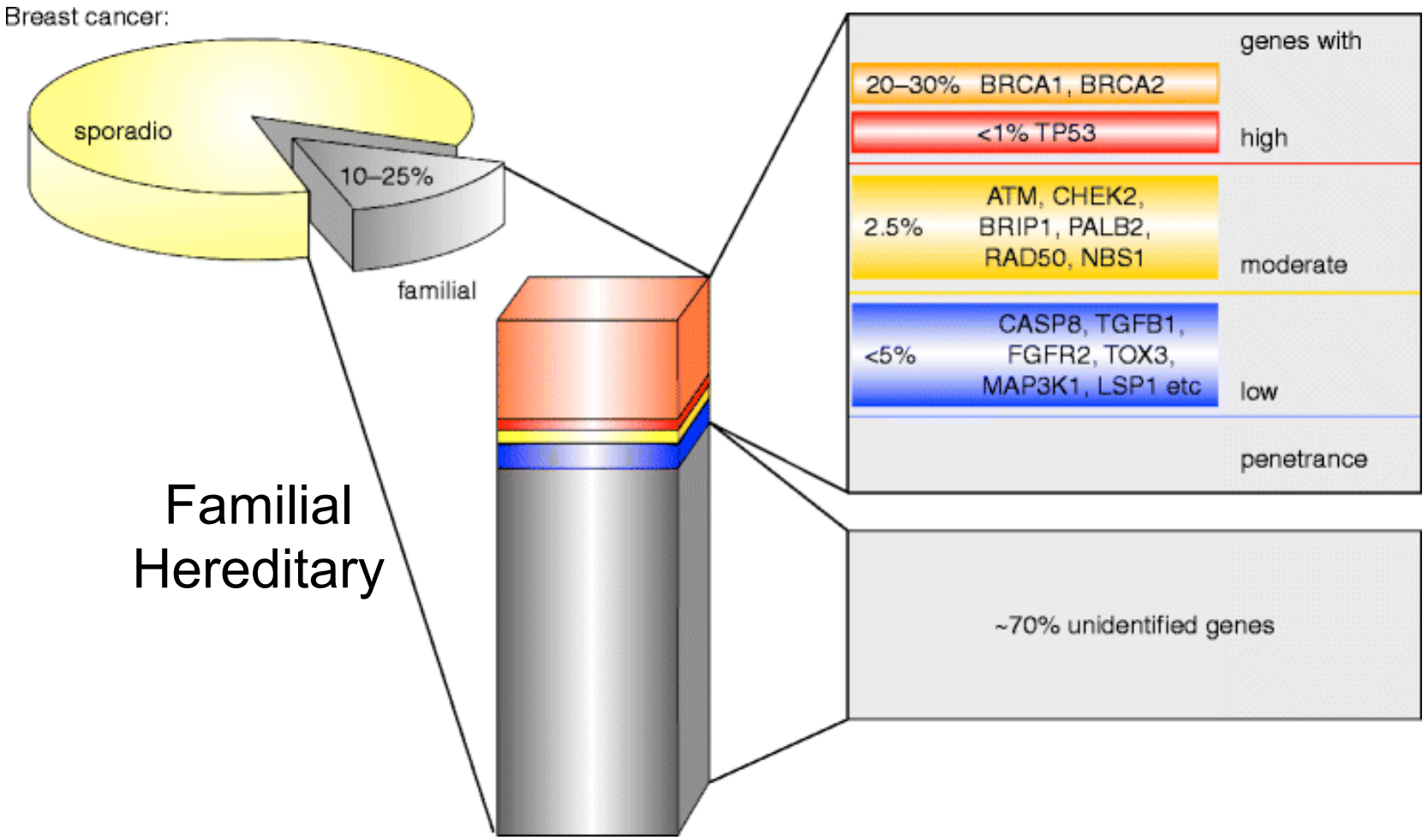


Ipermetilazione
del promotire
RB wt



Delezione regione o altri
meccanismi che
comportano LOH e
alterazione di RB wt

Breast cancer: susceptibility genes



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% within 5 years	21.1% within 10 yrs	10.8% within 10 yrs
		83% by age 70	62% by age 70
Ovarian	1%-2%	39%-63%	16.5%-27%
Male breast	0,10%	1,20%	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	1,60%		Elevated Risk

Quando si sospetta forma ereditaria: ricerca mutazioni in BRCA1 e BRCA2

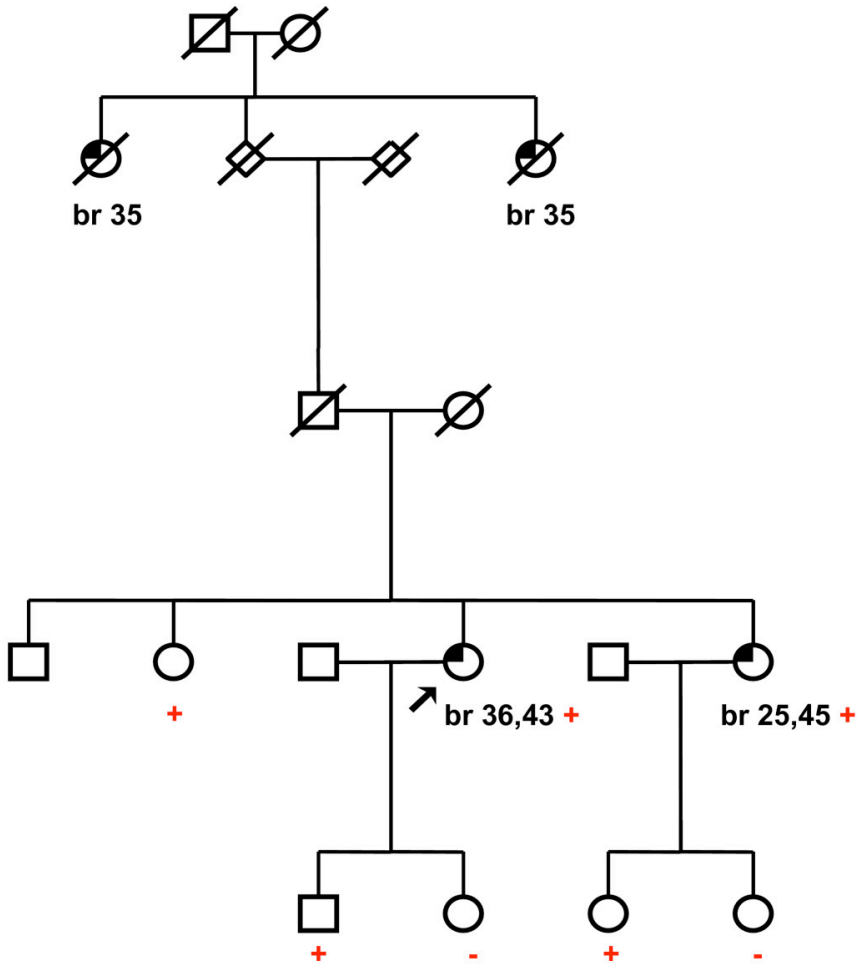
- 2 parenti di I grado affette da CM <50 anni;
- 3 parenti di I o II grado affette da CM indipendentemente dall'età alla diagnosi;
- 1 parente affetta da CM e >1 affetta da CO (relazione di parentela di I o II grado);
- 1 caso di CM bilaterale, indipendentemente da età;
- 2 parenti di I o di II grado affette da CO, indipendentemente da età;
- 1 caso di CM e CO sincroni o metacroni;
- 1 casi di CM maschile;
- 1 caso di CM femminile < 36 anni;
- 1 caso di CM femminile triplo-negativo e/o istotipo midollare < 50 anni.

CM: tumore mammella; CO: tumore ovarico

Tumore della mammella:
rischio (nelle femmine) di contrarre il tumore

Popolazione generale	1/12
Sorella con diagnosi a 65-70 anni	1/8
Sorella con diagnosi a 40 anni	1/4
Due consanguinei di I grado ammalati prima dei 40 anni	1/3

Componente genetica



Ricerca mutazioni: probanda

1) Identificazione mutazione (+)

- **Possibile estensione a familiari**

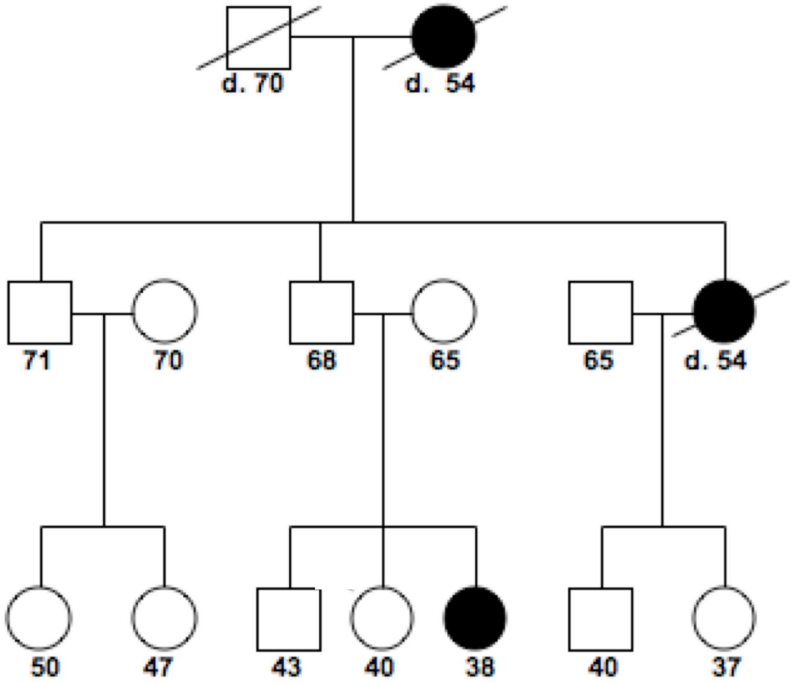
2) Nessuna alterazione

TEST NON INFORMATIVO: non consente di escludere la presenza di mutazioni.

- **Mutazione di BRCA1 e BRCA2 in regioni non analizzate;**
- **Mutazioni in altri geni associati a suscettibilità;**
- **Aggregazione familiare dovuta al caso o a origine multifattoriale;**

3) Identificazione di varianti di significato incerto

Indicate the genotype in the members of the family (breast cancer) only when it is certain



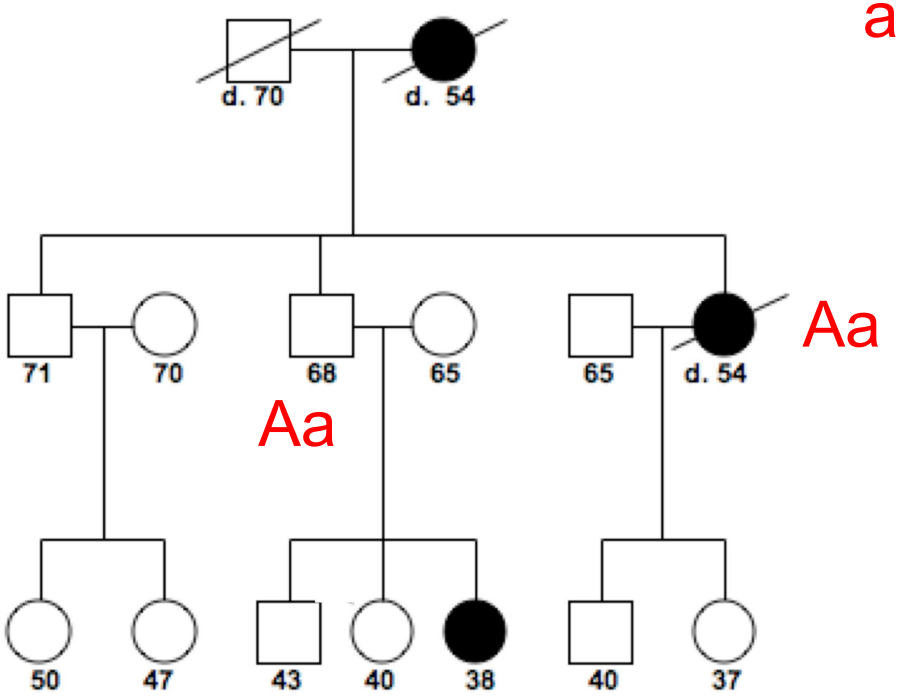
A = wt

a = mutated

Indicate the genotype in the members of the family (breast cancer) only when it is certain

Aa

A = wt
a = mutated



Aa

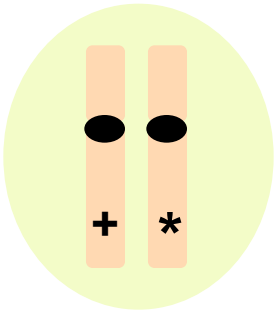
Aa

Aa

Geni di Risposta al danno del DNA

- **Riparano i danni al DNA**
- **Il cancro è causato da mutazioni inattivanti in entrambi gli alleli (considerati un sottogruppo di geni oncosoppressori)**
- **La perdita della loro funzione determina l'accumulo di mutazioni in altri geni cruciali**

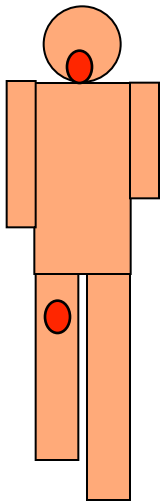
TUMORE EREDITARIO: 2 mutazioni germinali



Integrità del genoma
Riparazione del DNA

Mutazioni in entrambi gli alleli
(in tutte le cellule)

Evoluzione clonale
(una o più cellule)



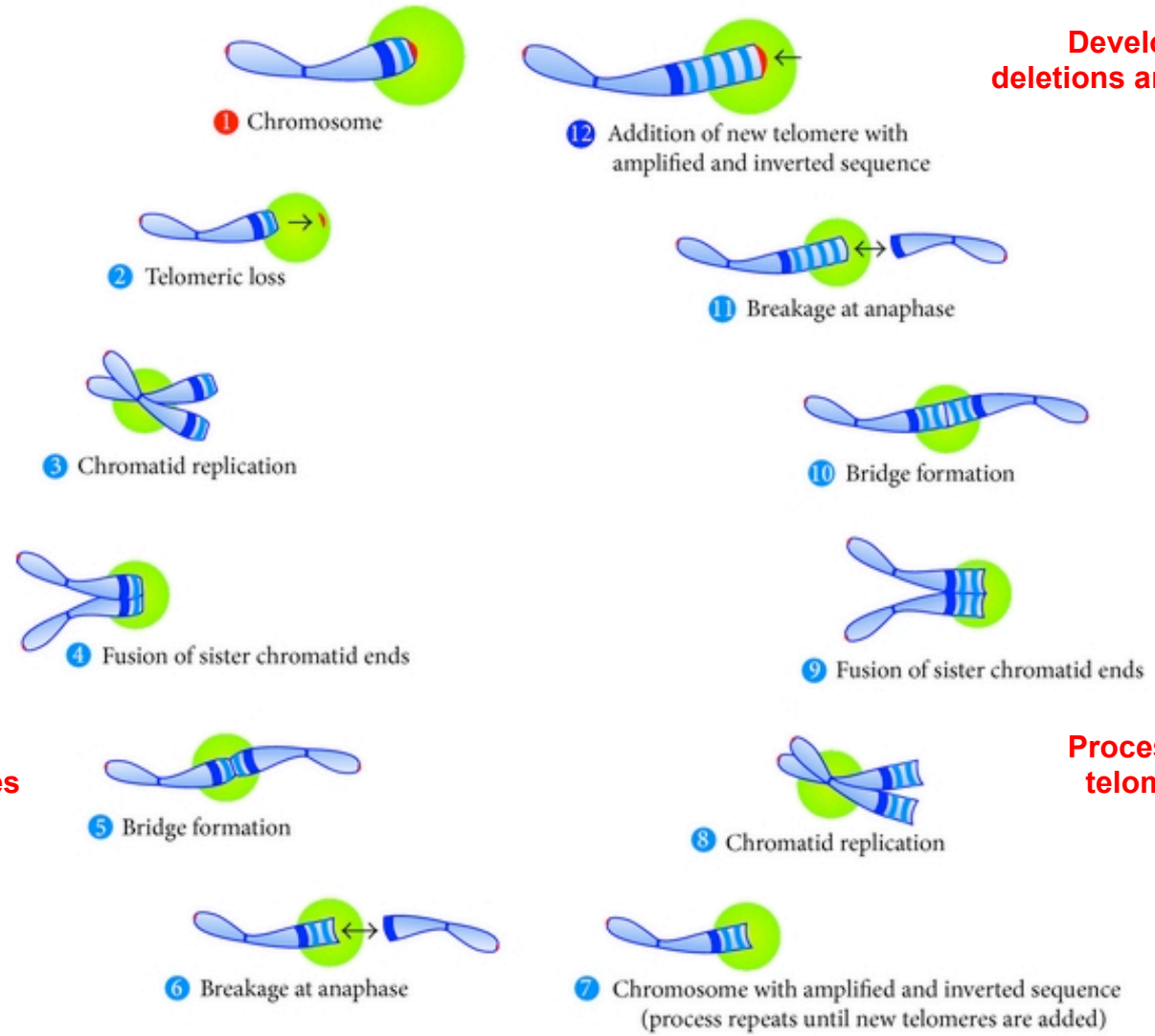
Gene	Malattia	Tipo di tumore
ATM	Atassia-Teleangiectasia	Linfomi
XPA, C,D,F	Xeroderma Pigmentosum	Tumori pelle
BLM	Bloom Syndrome	Tumori solidi
FANC A-T	Anemia di Fanconi	Leucemia, tumori solidi testa/collo

Heterogeneity in mutational spectrum
(somatic mutations in sporadic cancers)

In addition to point and chromosomal alterations, there are more complex mutational events:

- **Breakage-fusion-bridge cycles**: pancreatic cancers:
- **Balanced chains of somatic rearrangements**: prostate cancers:
- **Chromothripsis**: sarcomas and medulloblastoma
- **Kataegis**: breast cancers

Breakage-fusion-bridge (BFB) cycle



Developmental of deletions and amplifications

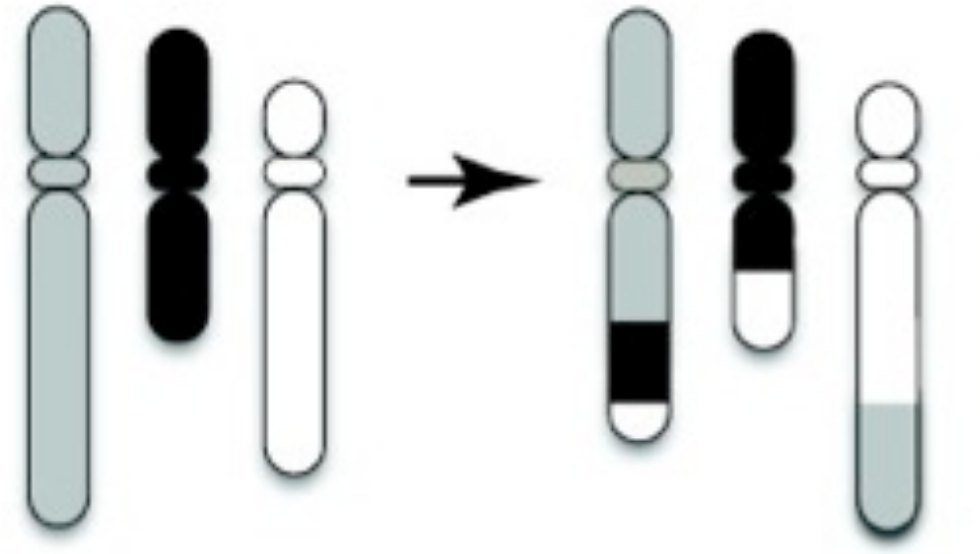
Dicentric chromosomes

Process repeated until telomere is restored

Break from being pulled to opposite directions

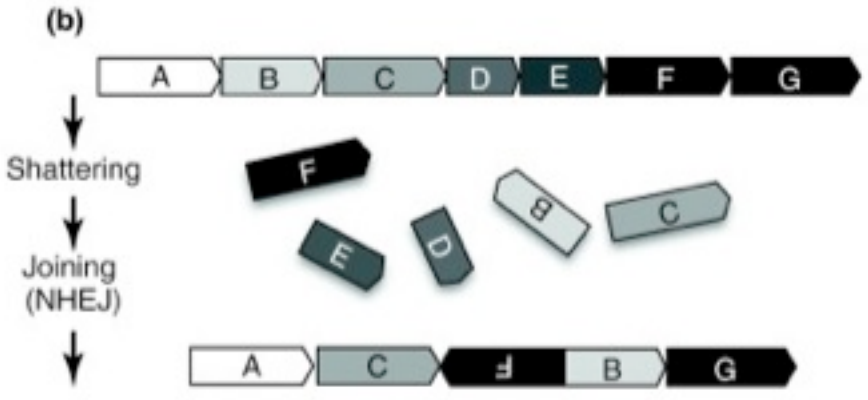
Balanced chains of somatically acquired genomic rearrangements

Up to ten genomic regions involved in a mutual exchange of DNA segments without copy number loss



Chromothripsis (2-3% of cancers)
catastrophic mutational process

A single event that causes genome shattering and reassembly, resulting in a characteristic pattern of oscillating copy number and up to several hundred genomic rearrangements localized to one or a few chromosomes.

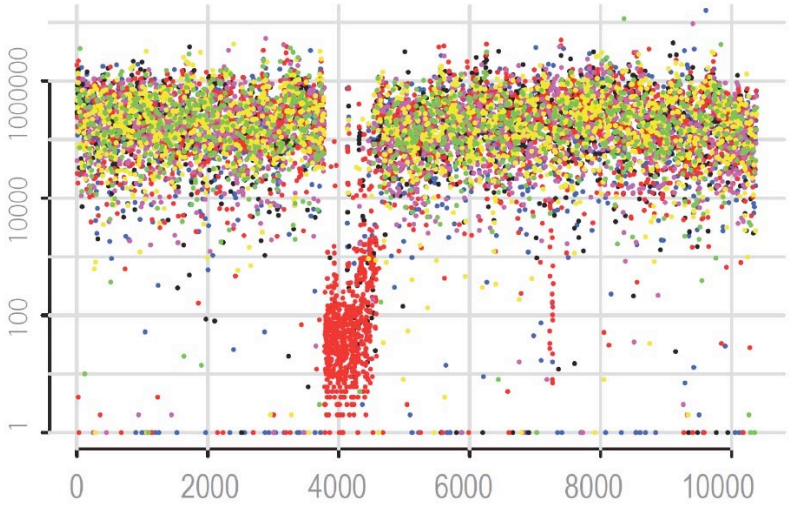


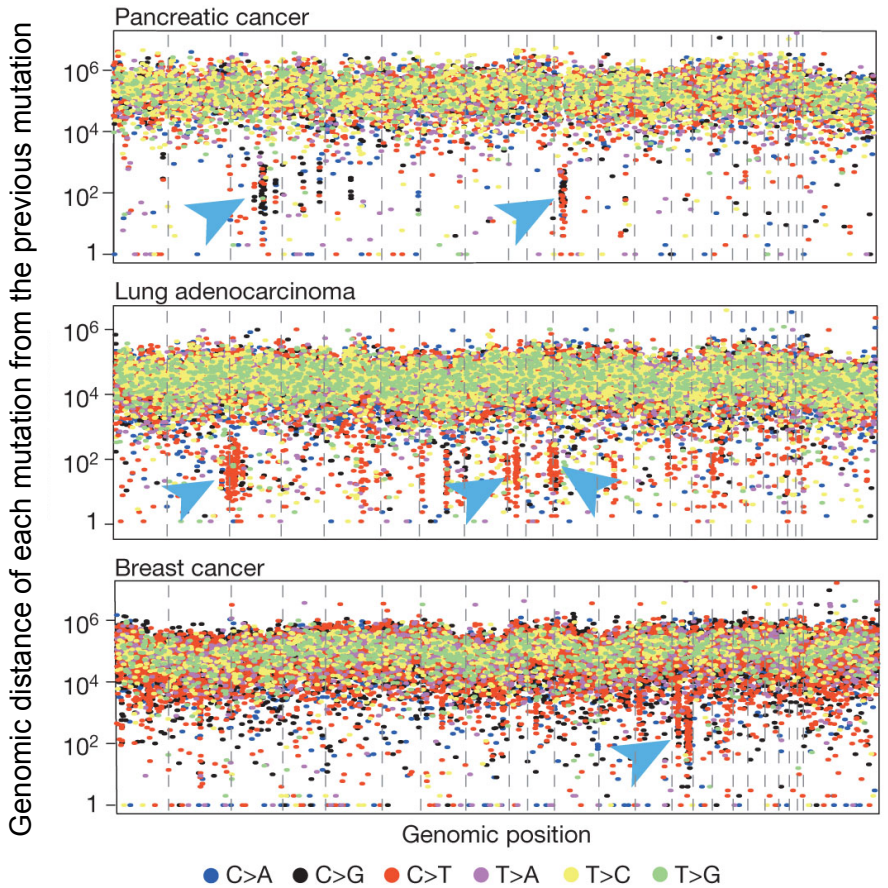
High rate in
Sarcomas
Medulloblastomas
AML

} in the presence of TP53 mutants

Kataegis (thunderstorm)

- Foci of localized substitution hypermutation (from few to several thousand)
- Often colocalization with somatic rearrangements (breaks have a role but are not sufficient in initiating the process)
- Almost C to T and/or C>G substitution in the context of a TpC dinucleotide
- Different genomic region in different cancers





Signatures of mutational processes in human cancer

Alexandrov et al. Nature 500, 415–421, 2013

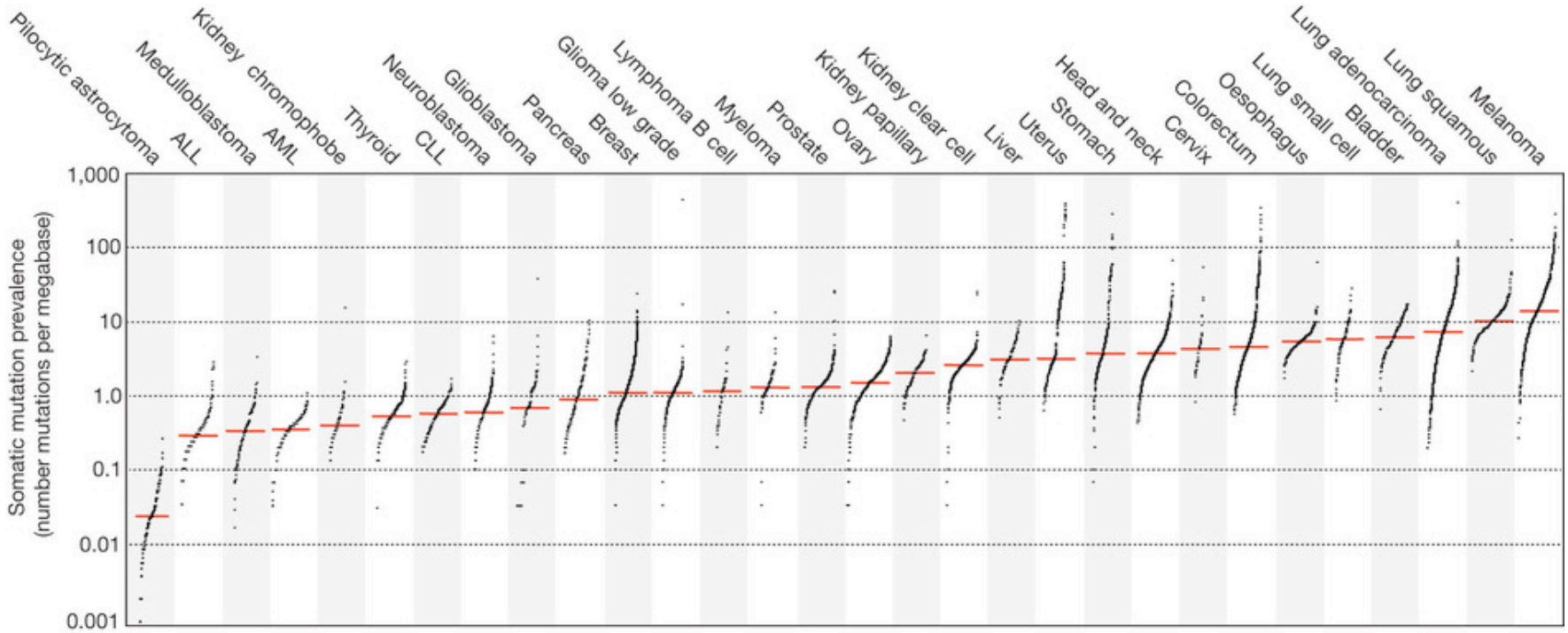
4,938,362 mutations

from 7,042 cancers

>20 distinct mutational signatures extracted

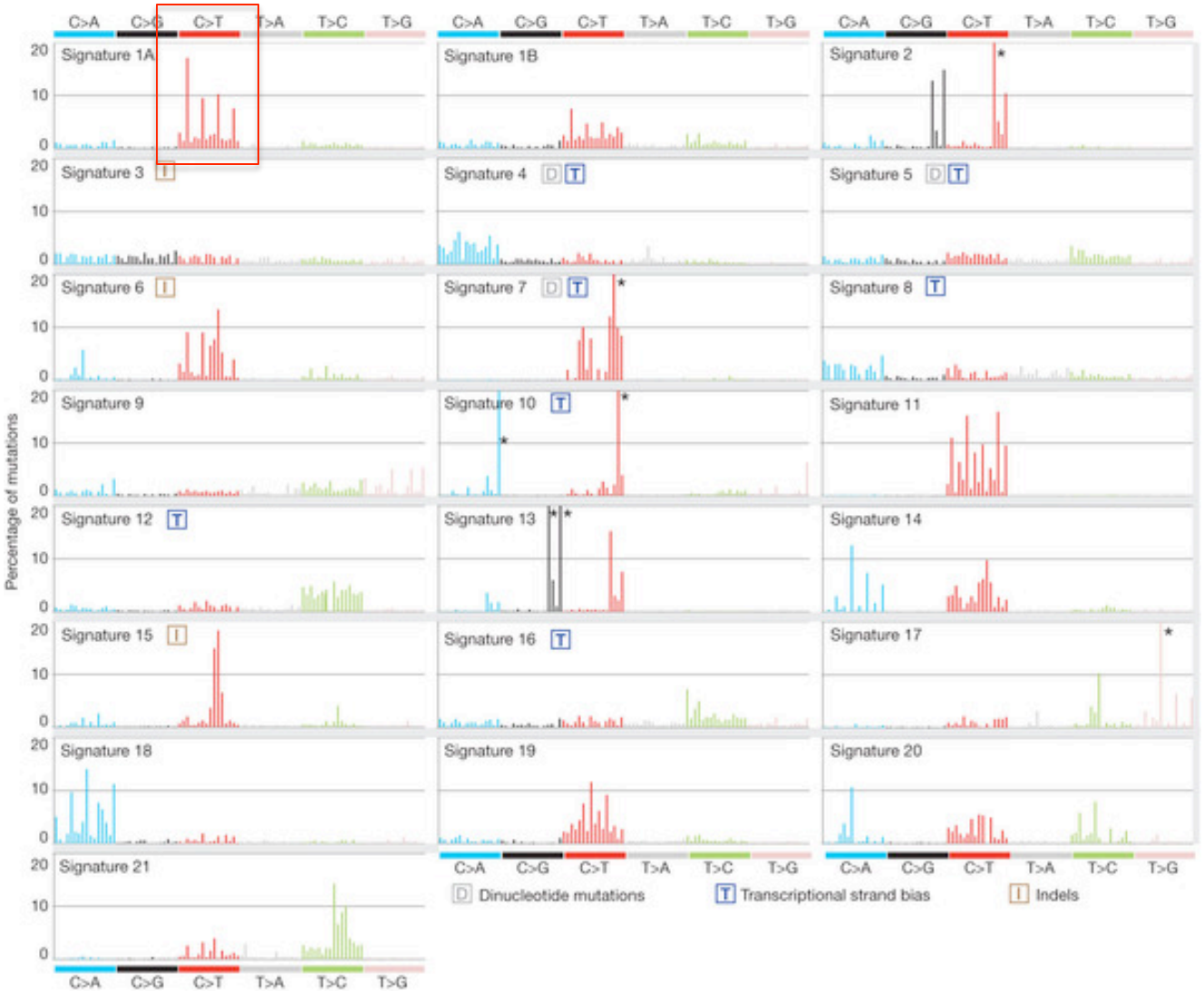
Mutational signature
Patterns of mutations that are characteristic of a type of cancer

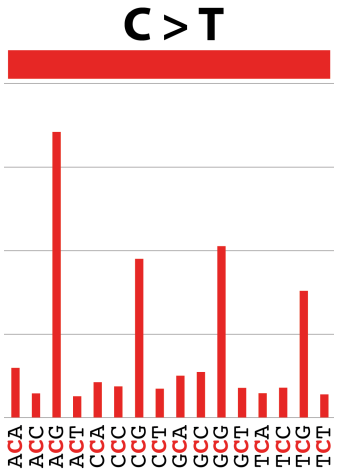
Number of mutations in different tumor samples



Mutational signatures

Six classes of base substitutions referred to by pyrimidine





C>T mutations in a CpG context Spontaneous deamination of methylated cytosines

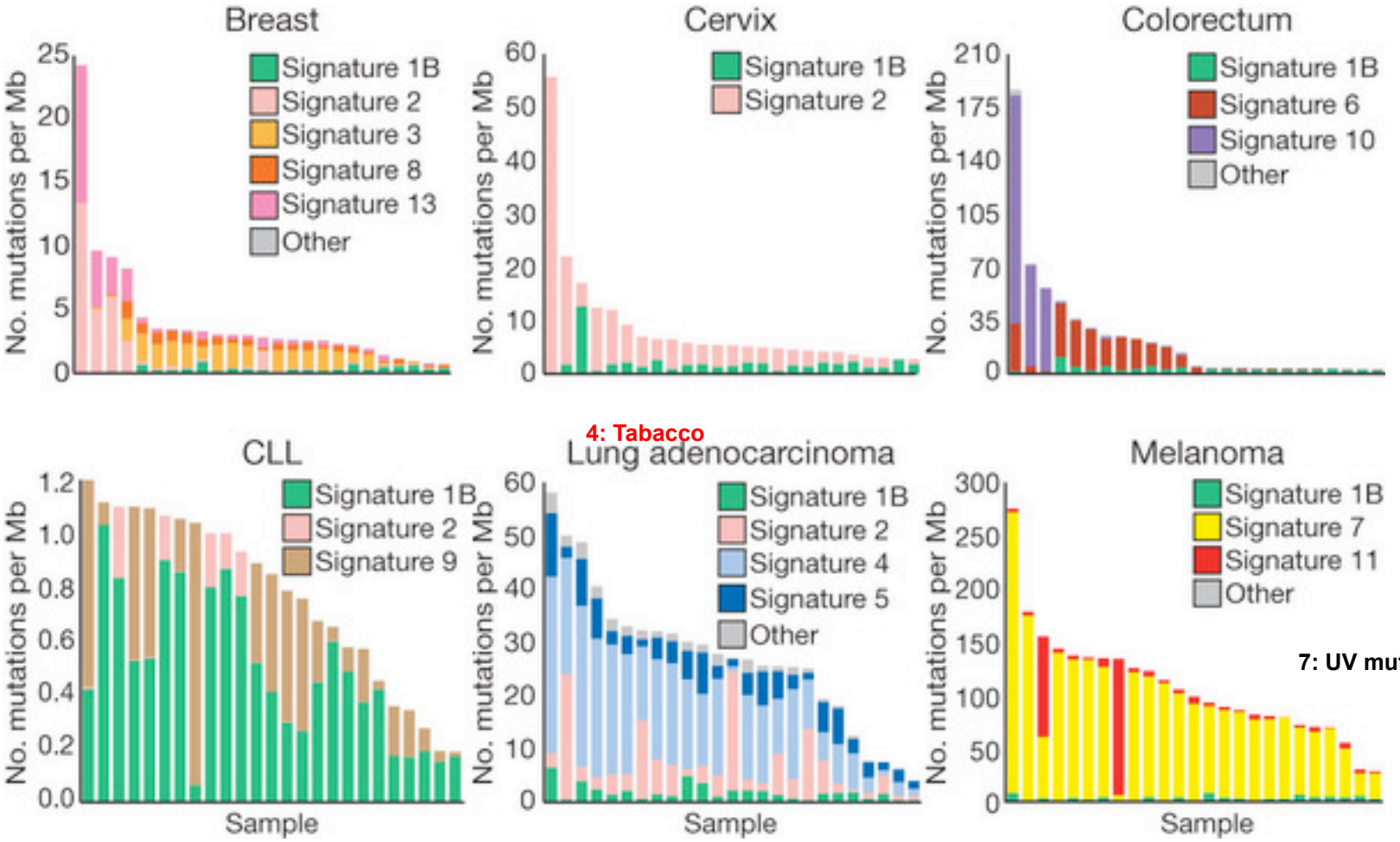
Mutational signature displayed using a 96 substitution classification defined by the substitution class (six: C>A, C>G, C>T, T>A, T>C and T>G) and the sequence context immediately 3' and 5' to the mutated base.

Signature in different cancers

2, 13: APOBEC Cytidine deaminase

3: DSB repair

6: mismatch repair



7: UV mutation