Mutazioni che interferiscono con il meccanismo di splicing

Table 16.1: Eleven ways to reduce or abolish the production of a functioning gene product

Change	Example
Delete:	
(i) the entire gene	Most α-thalassemia mutations (*Figure 16.3*)
(ii) part of the gene	60% of Duchenne muscular dystrophy (*Figure 16.2*)
Insert a sequence into the gene	Insertion of LINE-1 repetitive sequence (see Section 11.5.6) into *F8* gene in hemophilia A
Disrupt the gene structure:	
(i) by a translocation	X–autosome translocations in women with Duchenne muscular dystrophy (*Figure 14.10*)
(ii) by an inversion	Inversion in *F8* gene (*Figure 11.20*)
Prevent the promoter working:	
(i) by mutation	β-Globin g.-29A→G mutation (*Table 18.5*)
(ii) by methylation	*CDKN2A* gene in many tumors (Section 17.6.1)
Destabilize the mRNA:	
(i) polyadenylation site mutation	α-globin g.AATAAA→AATAGA mutation
(ii) by nonsense-mediated RNA decay	β-Globin p.Q39X
Prevent correct splicing (Section 11.4.3)	
(i) inactivate donor splice site	*PAX3* g.451+1G→T mutation (*Figure 16.1*)
(ii) inactivate acceptor splice site	*PAX3* g.452-2A→G mutation (*Figure 16.1*)
(iii) alter an exonic splicing enhancer	*SMN2* exon 7 g.C6T (Cartegni and Krainer 2002)
(iv) activate a cryptic splice site(maybe deep within an intron)	*LGMD2A* G624G (*Figure 11.12*)
	β-Globin IVS1–110G→A mutation (*Table 18.5*)
	CFTR 3849+10kb C→T (*Table 18.6*)
Introduce a frameshift in translation	*PAX3* g.874_875insG mutation (*Figure 16.1*)
Convert a codon into a stop codon	*PAX3* p.Q254X mutation (*Figure 16.1*)
Replace an essential amino acid	*PAX3* p.R271C mutation (*Figure 16.1*)
Prevent post-transcriptional processing	Cleavage-resistant collagen N-terminal propeptide in Ehlers Danlos VII syndrome (*Section 16.6.1*).
Prevent correct cellular localization of product	p.F508del mutation in cystic fibrosis

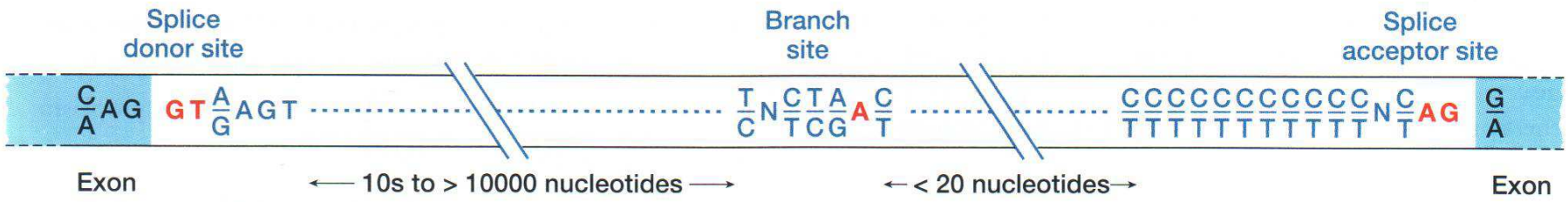
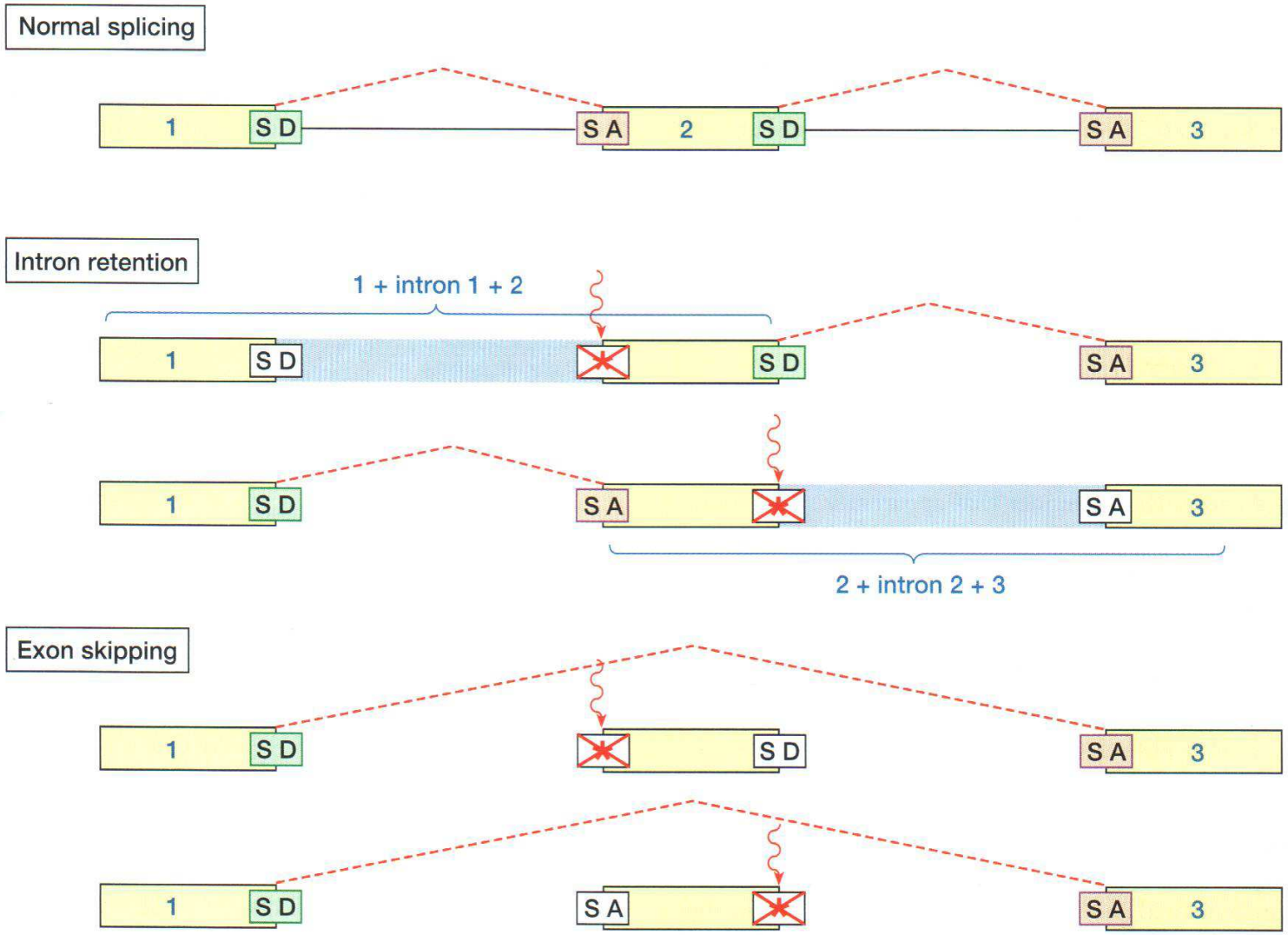


Figure 1.15: Consensus sequences at the DNA level for the splice donor, splice acceptor and branch sites in introns of complex eukaryotes.

Highlighted nucleotides are almost invariant (***note:*** rare ***AT–AC introns*** also exist where the conserved splice donor dinucleotide GT is replaced by AT and where the conserved splice acceptor dinucleotide AG is replaced by AC; see text). Other nucleotides represent the majority nucleotide found at this particular position, or equivalence between two nucleotides such as between C and T in the ***polypyrimidine tract*** adjacent to the 3′ end of the intron. Preferred motifs for the splice donor, branch site and splice acceptor in individual species (including human, *Drosophila melanogaster, Caenorhabditis elegans* and *Saccharomyces cerevisiae*) are listed by Lim and Burge (2001). Some other exonic and intronic sequences are known to regulate splicing including splice enhancer and splice silencer sequences (see Berget *et al.,* 1995). See also Fairbrother *et al.* (2002) for consensus sequences for exonic splicing enhancer sequences.

(A)


Normal splicing
1
S D
S A
2
S D
S A
3
Intron retention
1 + intron 1 + 2
1
S D
S D
S A
3
1
S D
S A
S A
3
2 + intron 2 + 3
Exon skipping
1
S D
S D
S A
3
1
S D
S A
S A
3

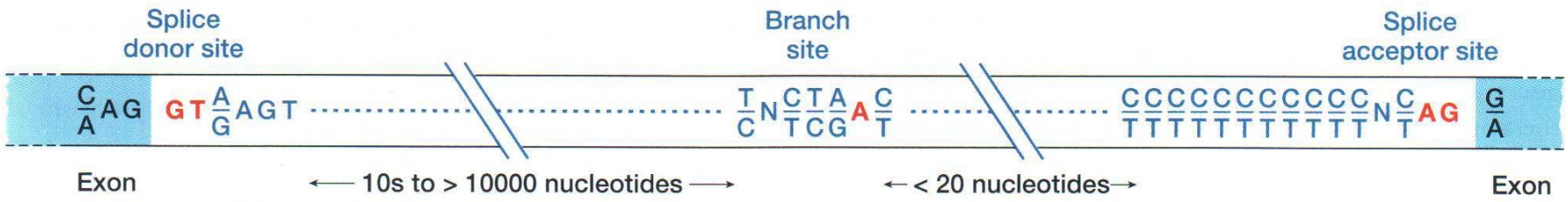


Figure 1.15: Consensus sequences at the DNA level for the splice donor, splice acceptor and branch sites in introns of complex eukaryotes.

Highlighted nucleotides are almost invariant (***note:*** rare ***AT–AC introns*** also exist where the conserved splice donor dinucleotide GT is replaced by AT and where the conserved splice acceptor dinucleotide AG is replaced by AC; see text). Other nucleotides represent the majority nucleotide found at this particular position, or equivalence between two nucleotides such as between C and T in the ***polypyrimidine tract*** adjacent to the 3′ end of the intron. Preferred motifs for the splice donor, branch site and splice acceptor in individual species (including human, *Drosophila melanogaster, Caenorhabditis elegans* and *Saccharomyces cerevisiae*) are listed by Lim and Burge (2001). Some other exonic and intronic sequences are known to regulate splicing including splice enhancer and splice silencer sequences (see Berget *et al.,* 1995). See also Fairbrother *et al.* (2002) for consensus sequences for exonic splicing enhancer sequences.

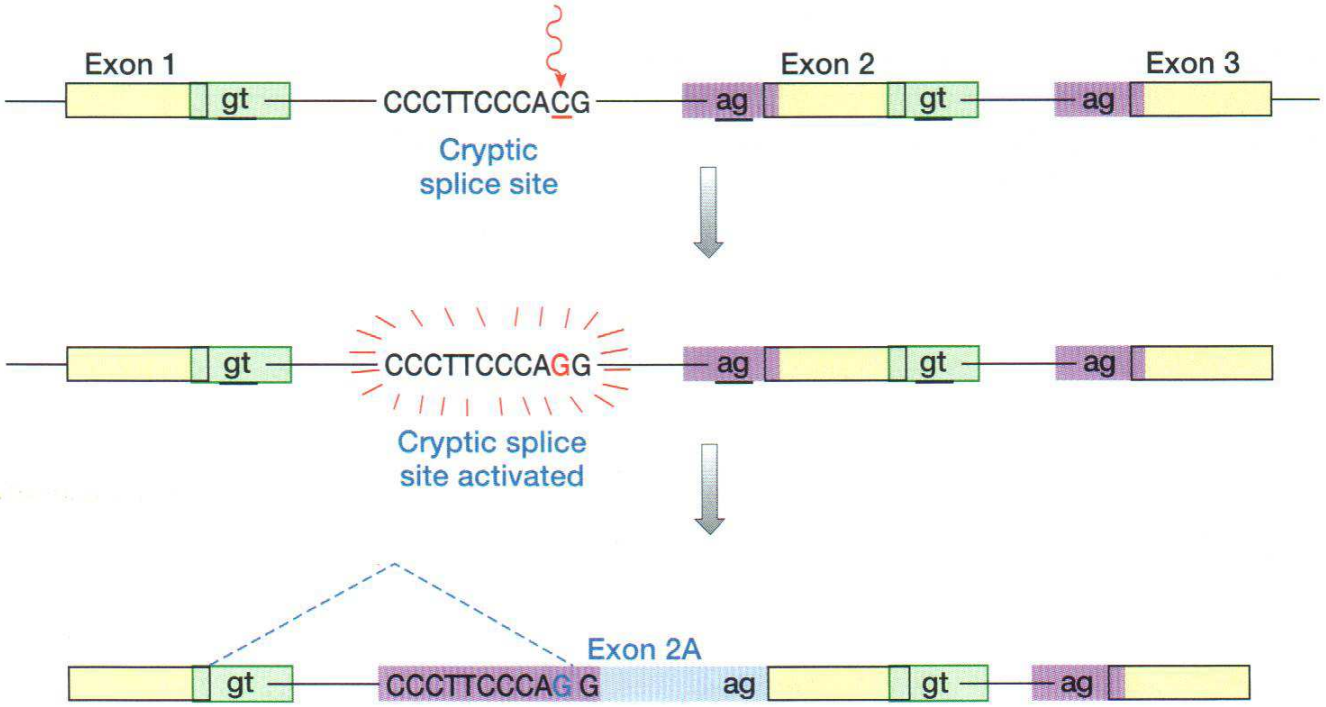


Figure 11.13: Mutations can cause abnormal RNA splicing by activation of cryptic splice sites.

Activation of a cryptic splice acceptor sequence located within an intron (compare *Figure 11.12* which illustrates activation of a cryptic splice donor site within an exon). A mutation can result in the alteration of a sequence which is not important for RNA splicing so as to create a new, alternative splice site. In the example illustrated, the mutation is envisaged to change a single nucleotide in intron 1. The nucleotide happens to occur within a cryptic splice site sequence that is closely related to the splice acceptor consensus sequence (see *Figure 1.15*), but does not have the conserved AG dinucleotide. The mutation overcomes this difference, activating the cryptic splice site so that it competes with the natural splice acceptor site. If it is used by the splicing apparatus, a novel exon, exon 2A, results, which contains additional sequence which may or may not result in a frameshift.

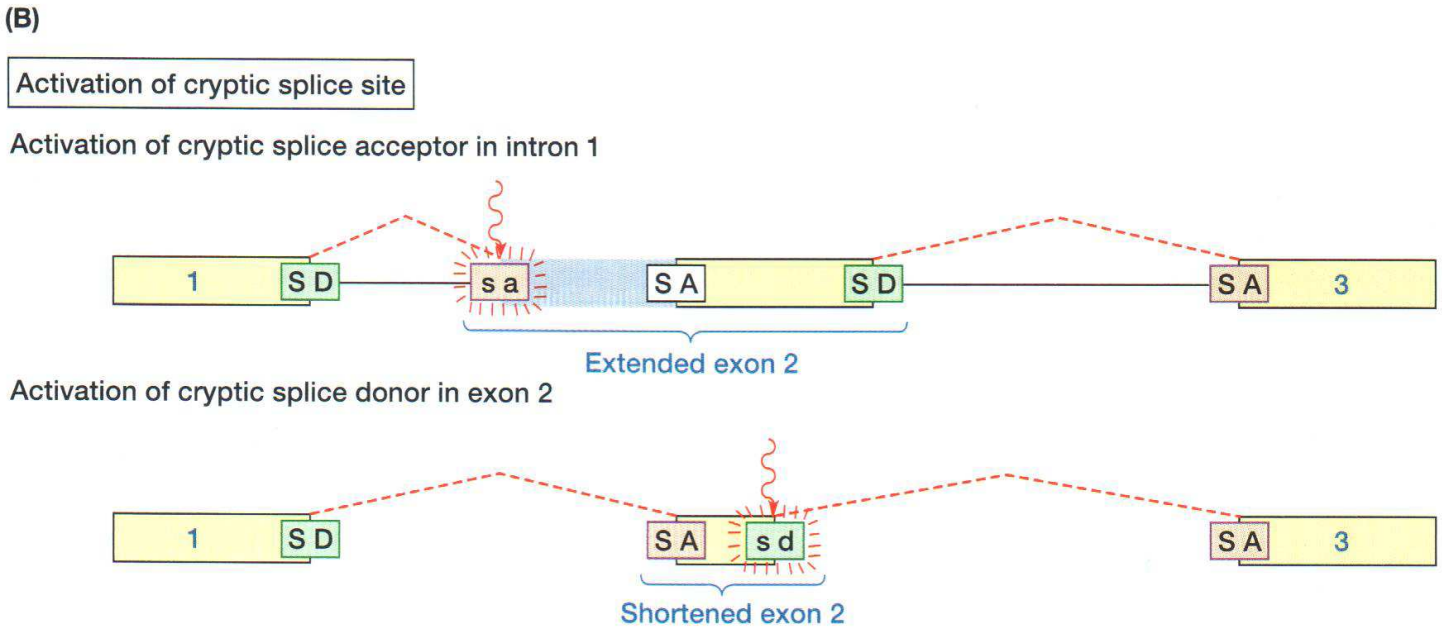


Figure 11.11: Splicing mutations arise by alteration of conserved splice signals or by activation of cryptic splice sites.

(A) ***Alteration of conserved splice signals.*** Mutation at splice donor or splice acceptor sequences (see *Figure 1.15* for consensus sequences) can result in: (a) intron retention where there is failure of splicing and an intervening intron sequence is not excised; (b) exon skipping where the spliceosome brings together the splice donor and splice acceptor sites of non-neighboring exons. ***Note:*** a splice site mutation may occasionally cause indirect activation of an alternative cryptic splice site (not normally used in splicing), in preference to using another legitimate splice site – see e.g. Takahara *et al.*, 2002). **(B)** ***Direct activation of cryptic splice sites.*** A mutation can directly activate a cryptic splice site by changing its sequence so that it becomes more like the consensus splice donor or acceptor sequence. The *altered* cryptic splice site can now be recognized and used by the spliceosome. See *Figures 11.12* and *11.13* for examples of activation of an exonic and an intronic cryptic splice site, respectively.

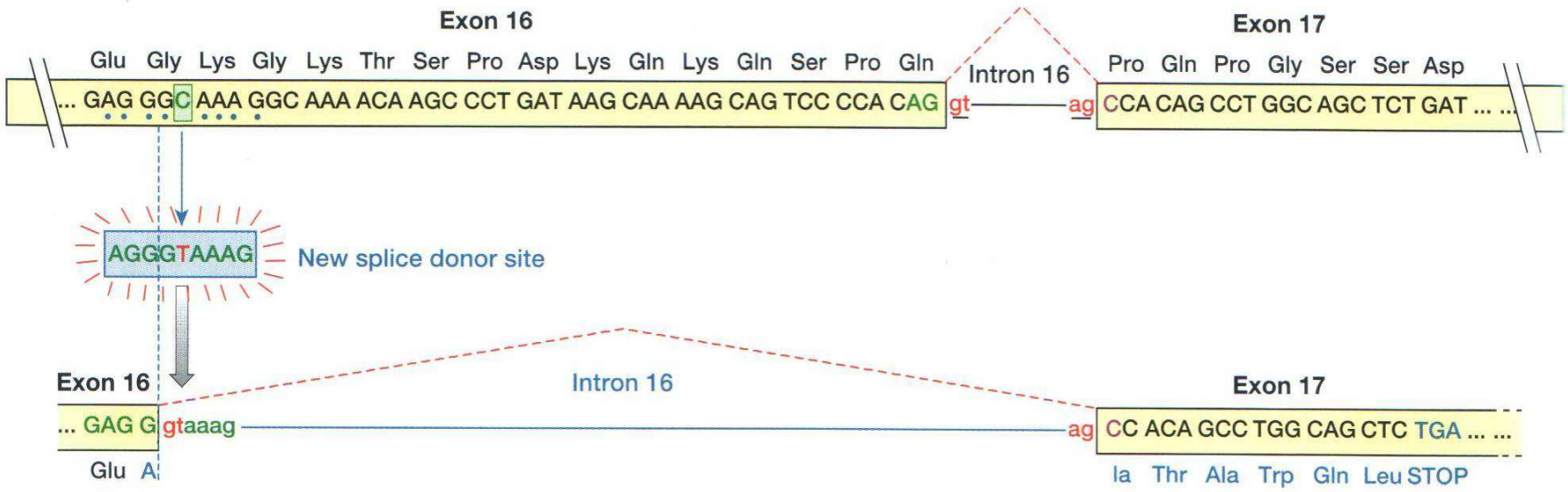


Figure 11.12: When a silent mutation is not silent.

This example shows a mutation that was identified in a LGMD2A limb girdle muscular dystrophy patient. The mutation was found in the calpain 3 gene, a known locus for this form of muscular dystrophy, but occurred at the third base position of a codon and appeared to be a silent mutation. It would lead to replacement of one glycine codon (GGC) by another glycine codon (GGT). However, the mutation is believed nevertheless to be pathogenic. The substitution results in activation of a cryptic splice donor sequence (AGGGCAAAAG) within exon 16 resulting in aberrant splicing with the loss of coding sequence from exon 16 and the introduction of a frameshift. See Richard and Beckmann (1995). ***Note:*** another possibility for pathogenic synonymous mutations are ones which cause their effect by mutating an exonic splice enhancer sequence (Section 11.4.3).

In alcuni casi mutazioni “loss of function” possono essere dominanti:

- Insufficienza aplotipica
- Sensibilità al dosaggio genico
- Mutazioni dominanti negative

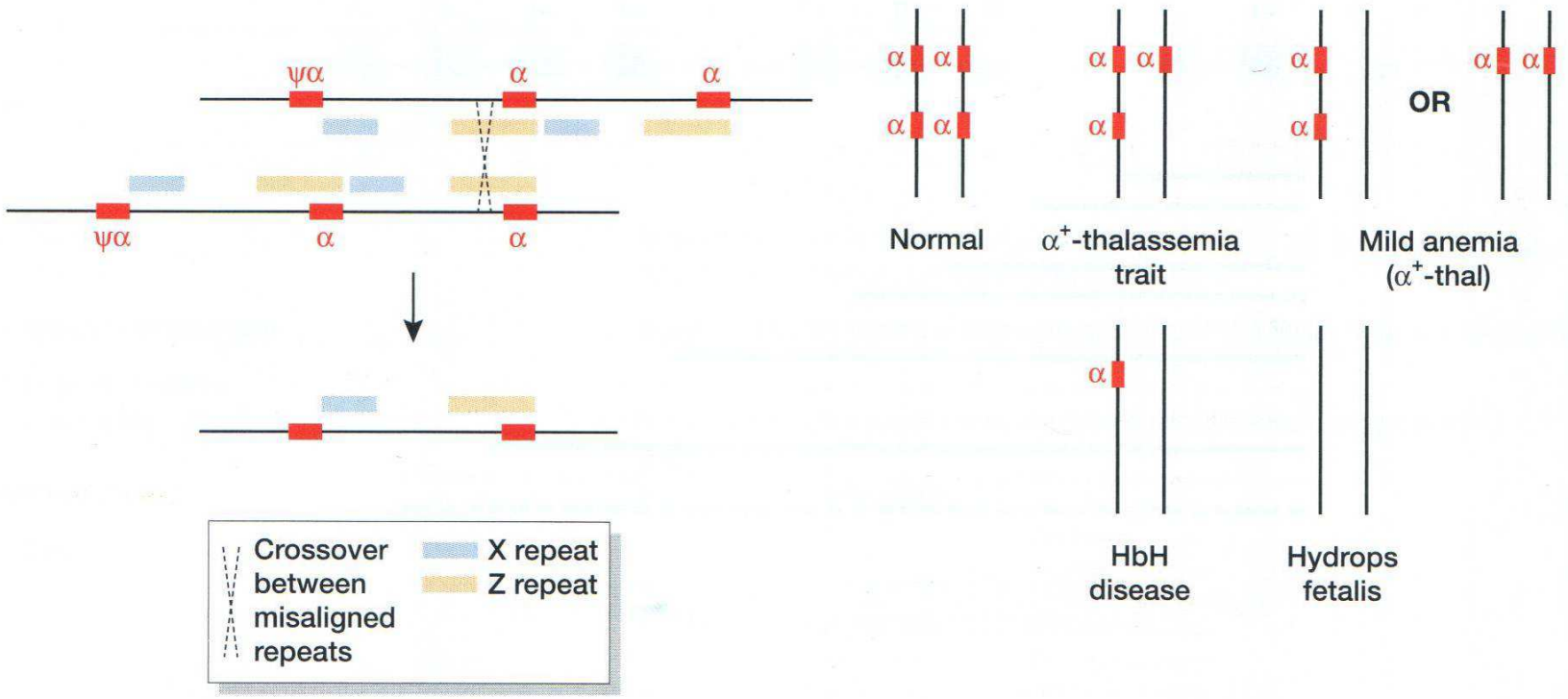


Figure 16.3: Deletions of α-globin genes in α-thalassemia.

Normal copies of chromosome 16 carry two active α-globin genes and an inactive pseudogene arranged in tandem. Repeat blocks (labeled X and Z) may misalign, allowing unequal crossover. The diagram shows unequal crossover between misaligned Z repeats producing a chromosome carrying only one active α gene. Unequal crossovers between X repeats have a similar effect. Unequal crossovers between other repeats (not shown) can produce chromosomes carrying no functional α gene. Individuals may thus have any number from 0 to 4 or more α-globin genes. The consequences become more severe as the number of α genes diminishes. See Weatherall *et al.* (Further reading) for details.

Table 4.2 **Eye Pigment Quantification for *Drosophila* White Alleles**

Genotypes	Relative Amount of Total Pigment
w^+/w^+ (wild type)	1.0000
w/w (white)	0.0044
w^t/w^t (tinged)	0.0062
w^a/w^a (apricot)	0.0197
w^{bl}/w^{bl} (blood)	0.0310
w^e/w^e (eosin)	0.0324
w^{ch}/w^{ch} (cherry)	0.0410
w^{a3}/w^{a3} (apricot-3)	0.0632
w^w/w^w (wine)	0.0650
w^{co}/w^{co} (coral)	0.0798
w^{sat}/w^{sat} (satsuma)	0.1404
w^{col}/w^{col} (colored)	0.1636

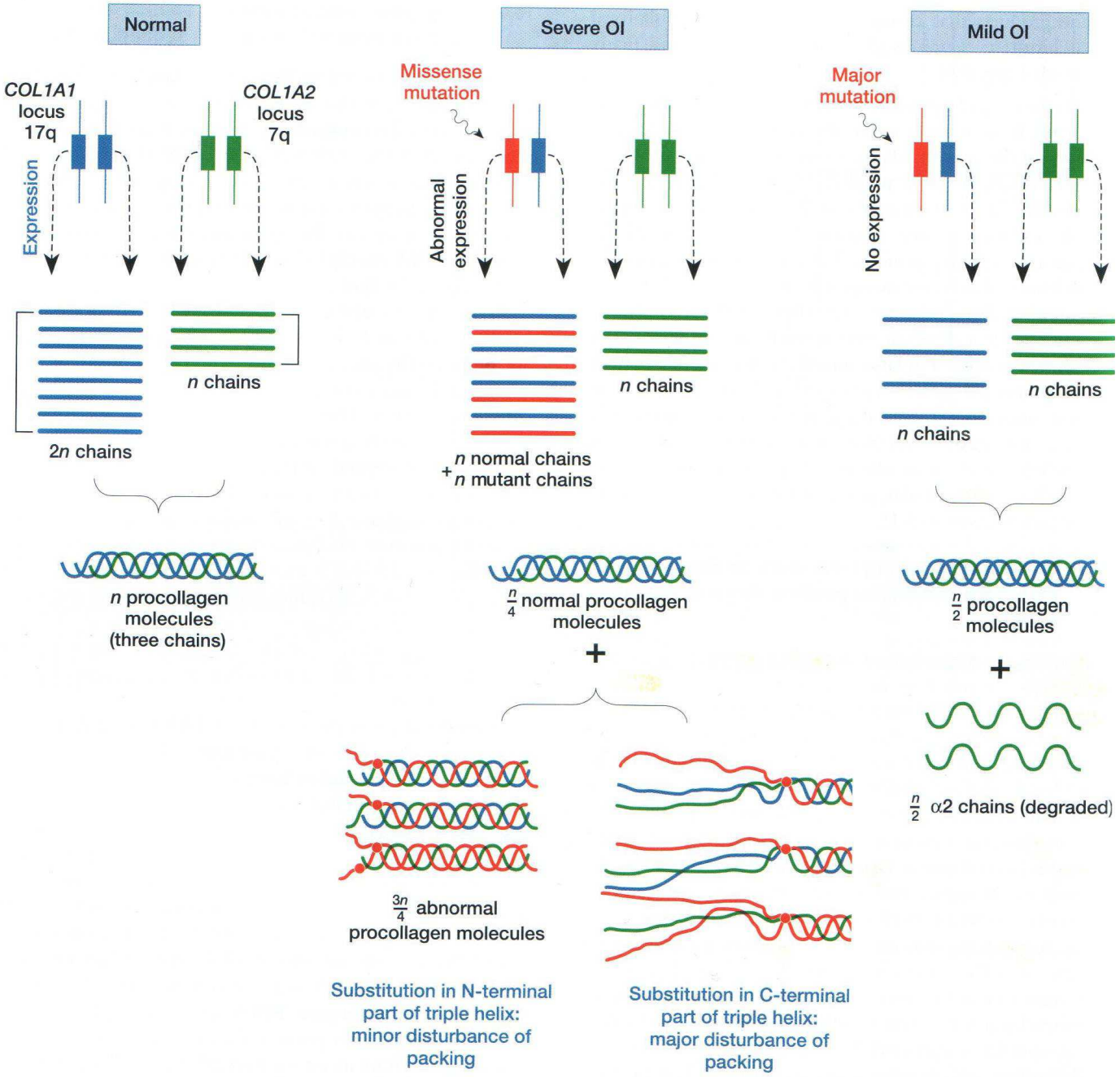
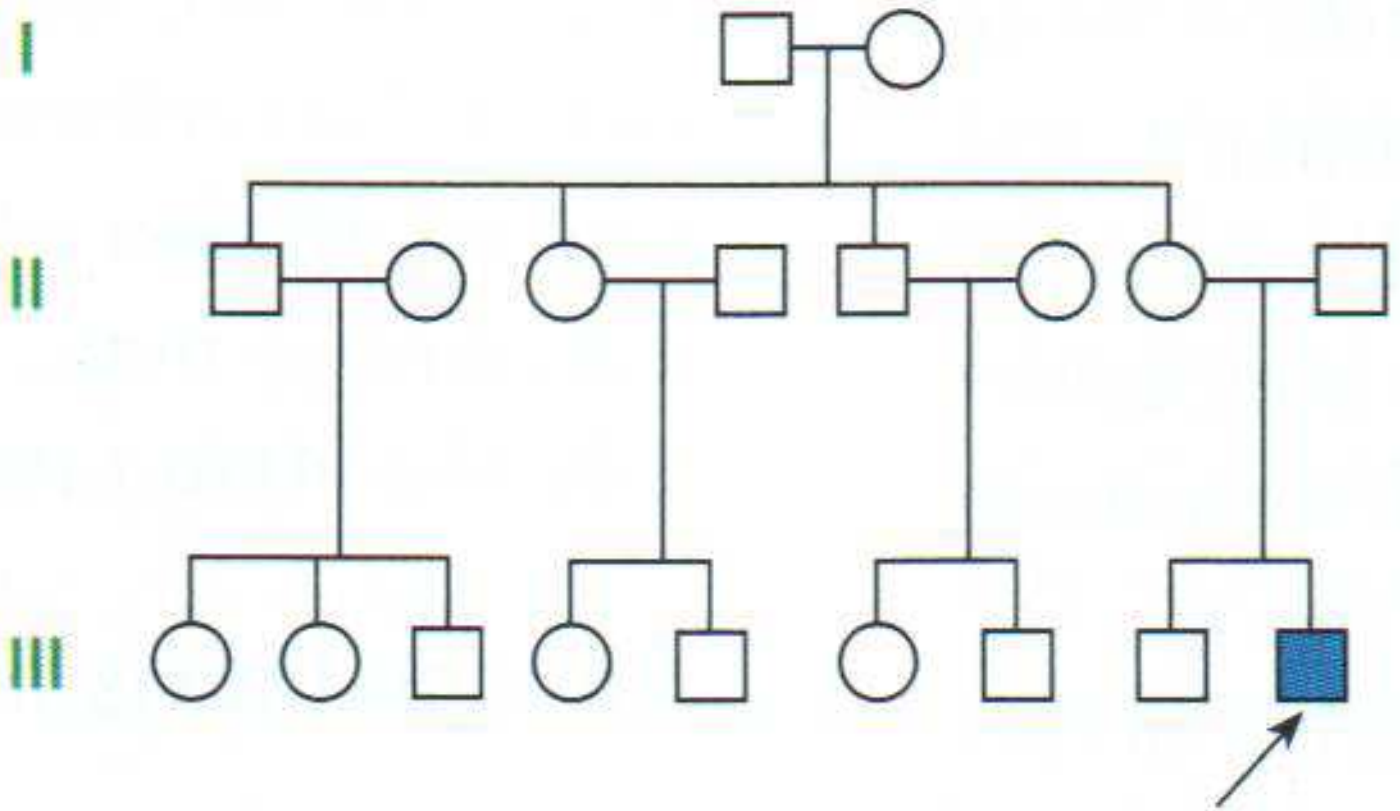
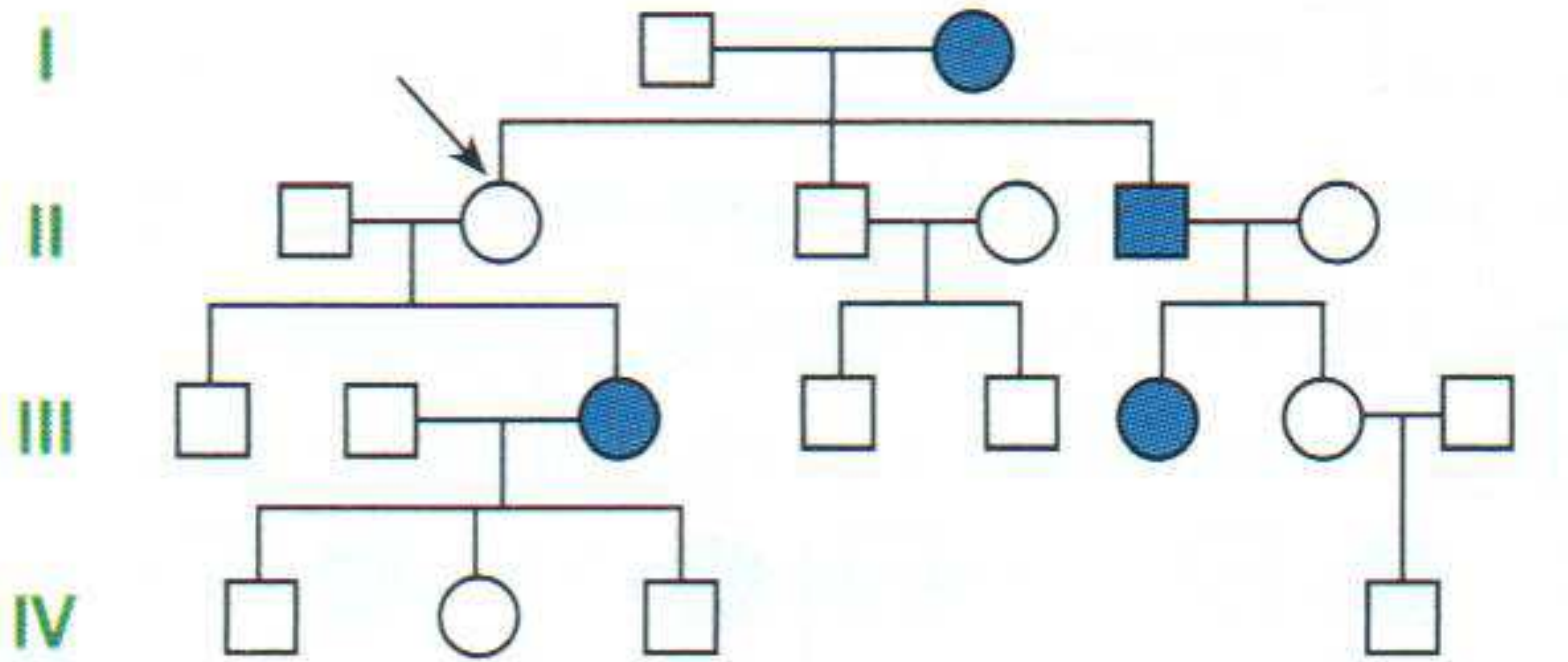

Normal
Severe OI
Mild OI
COL1A1
locus
17q
COL1A2
locus
7q
Expression
Missense
mutation
Abnormal
expression
Major
mutation
No expression
n chains
2n chains
n chains
n normal chains
+ n mutant chains
n chains
n chains
n procollagen
molecules
(three chains)
n/4 normal procollagen
molecules
+
n/2 procollagen
molecules
+
n/2 α2 chains (degraded)
3n/4 abnormal
procollagen molecules
Substitution in N-terminal
part of triple helix:
minor disturbance of
packing
Substitution in C-terminal
part of triple helix:
major disturbance of
packing

Table 16.3: Mechanisms of gain of function mutations

Malfunction	Gene	Disease	MIM no.
Overexpression	*PMP22*	Charcot–Marie–Tooth disease	118200
Receptor permanently 'on'	*GNAS*	McCune–Albright disease	174800
Acquire new substrate (Pittsburgh allele)	*PI*	α_1-Antitrypsin deficiency	107400
Ion channel inappropriately open	*SCN4A*	Paramyotonia congenita	168300
Structurally abnormal multimers	*COL2A1*	Osteogenesis imperfecta	Various
Protein aggregation	*HD*	Huntington disease	143100
Chimeric gene	*BCR–ABL*	Chronic myeloid leukemia	151410


I
II
III


I
II
III
IV

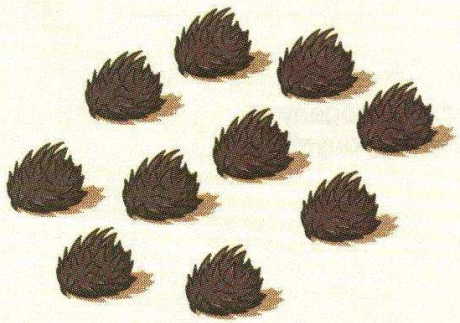
Penetranza la **frequenza** (in percentuale) con cui un allele si manifesta fenotipicamente in individui in una popolazione

Espressività il **grado** in cui un genotipo si manifesta fenotipicamente in un individuo

a)

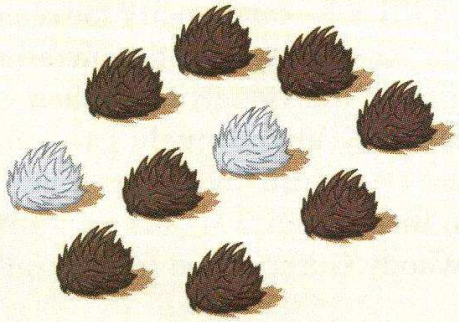
Complete penetrance

Identical known genotypes yield 100% expected phenotype.



Incomplete penetrance

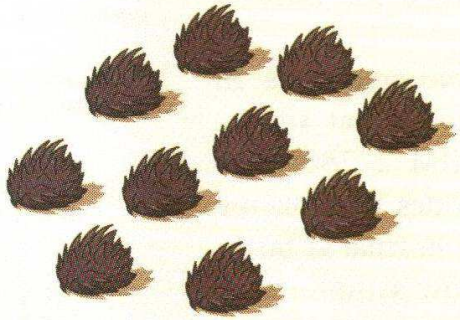
Identical known genotypes yield <100% expected phenotype.



b)

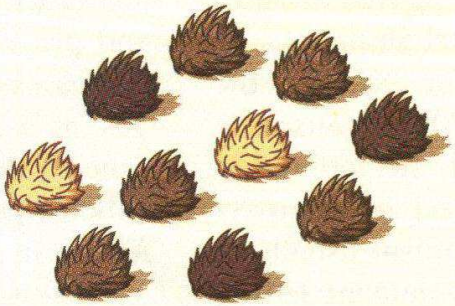
Constant expressivity

Identical known genotypes with no expressivity effect yield 100% expected phenotype.



Variable expressivity

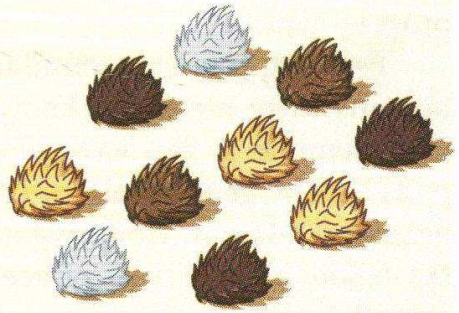
Identical known genotypes with an expressivity effect yield a range of phenotypes.



c)

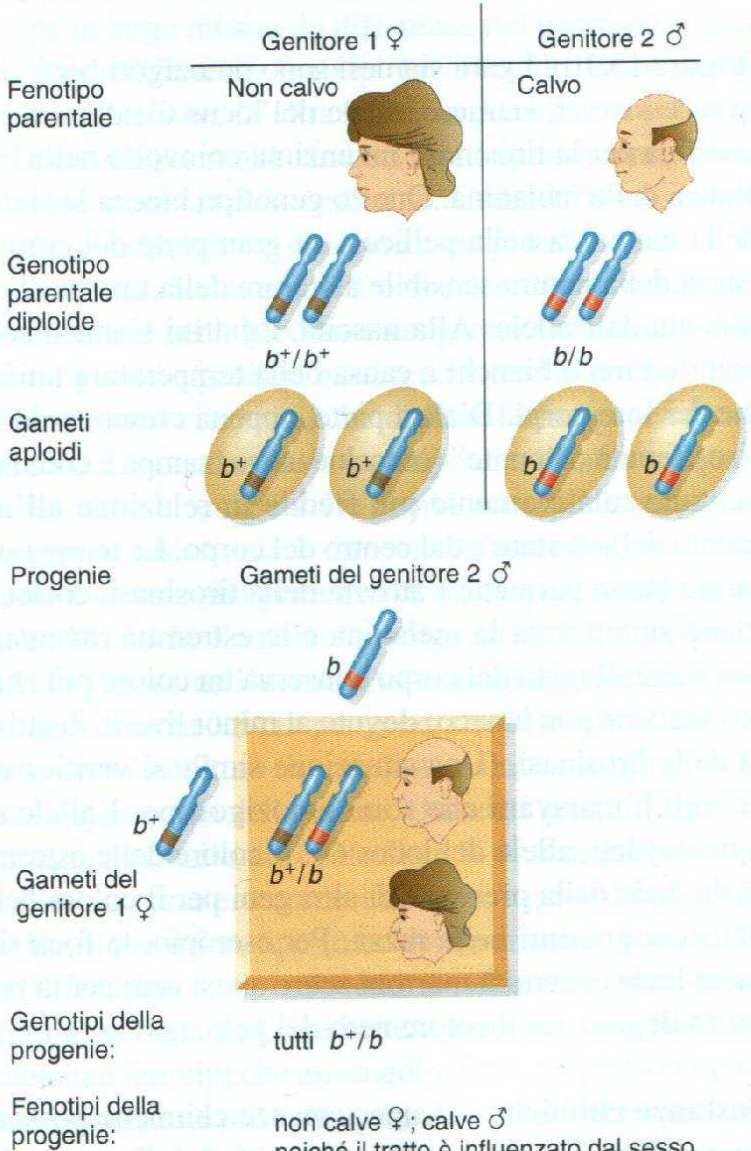
Incomplete penetrance with variable expressivity

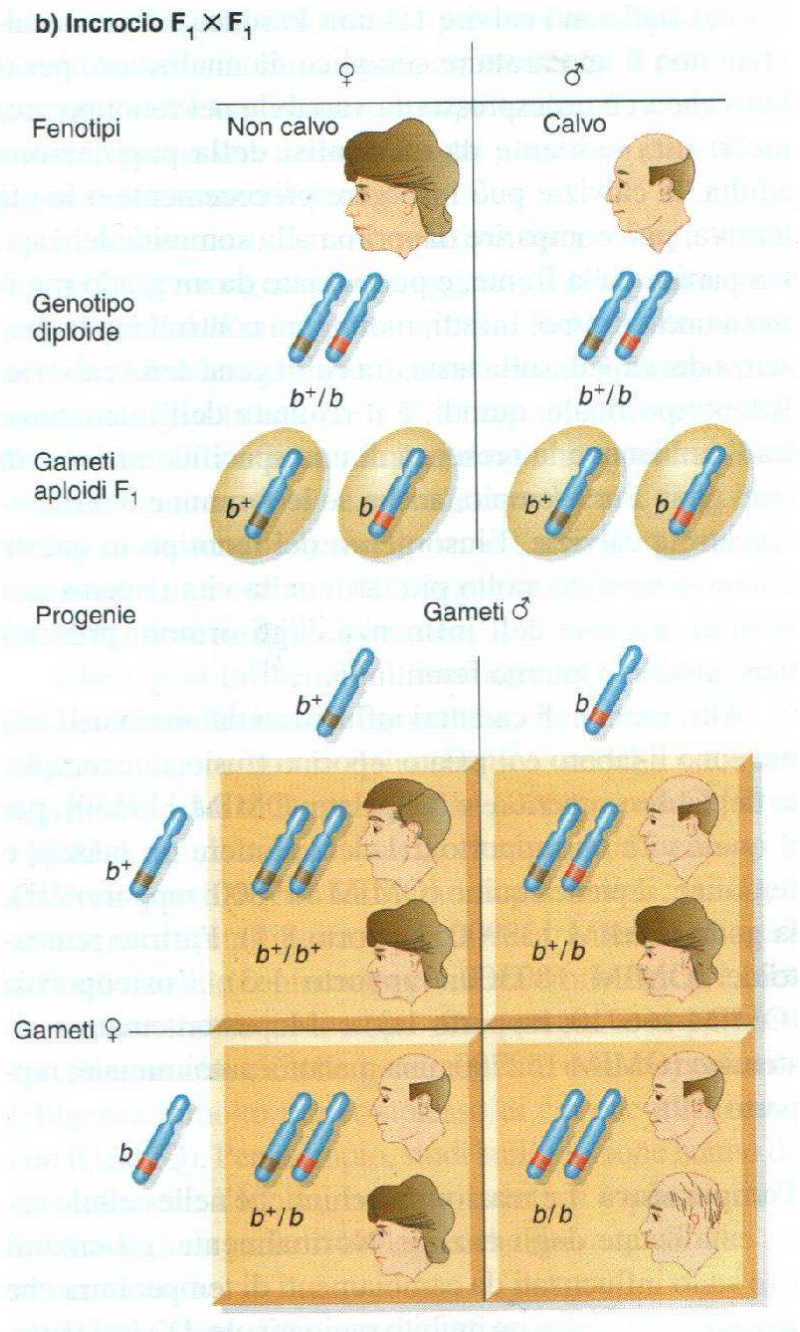
Identical known genotypes produce a broad range of phenotypes due to varying degrees of gene activation and expression.



- Età di insorgenza
- Sesso
- Temperatura
- Dieta

a) Femmina non calva × maschio calvo *b*/*b*




b) Incrocio $F_1 \times F_1$
♀
♂
Fenotipi
Non calvo
Calvo
Genotipo diploide
b^+/b
b^+/b
Gameti aploidi F_1
b^+
b
b^+
b
Progenie
Gameti ♂
b^+
b
b^+
b^+/b^+
b^+/b
Gameti ♀
b
b^+/b
b/b

	Maschi	Femmine
Labbro leporino	2	1
Gotta	8	1
Artrite reumatoide	1	3
Osteoporosi	1	3
LES	1	9

Figure 4.12

Effect of temperature on gene expression. **(a)** Himalayan rabbit. **(b)** White extremities result when a Himalayan rabbit is reared at above 30°C. **(c)** Normal Himalayan pattern when rabbit is reared at 25°C. **(d)** Normal Himalayan pattern when rabbit is reared at 25°C, with a dark patch on the side where the flank has been cooled to below 25°C.

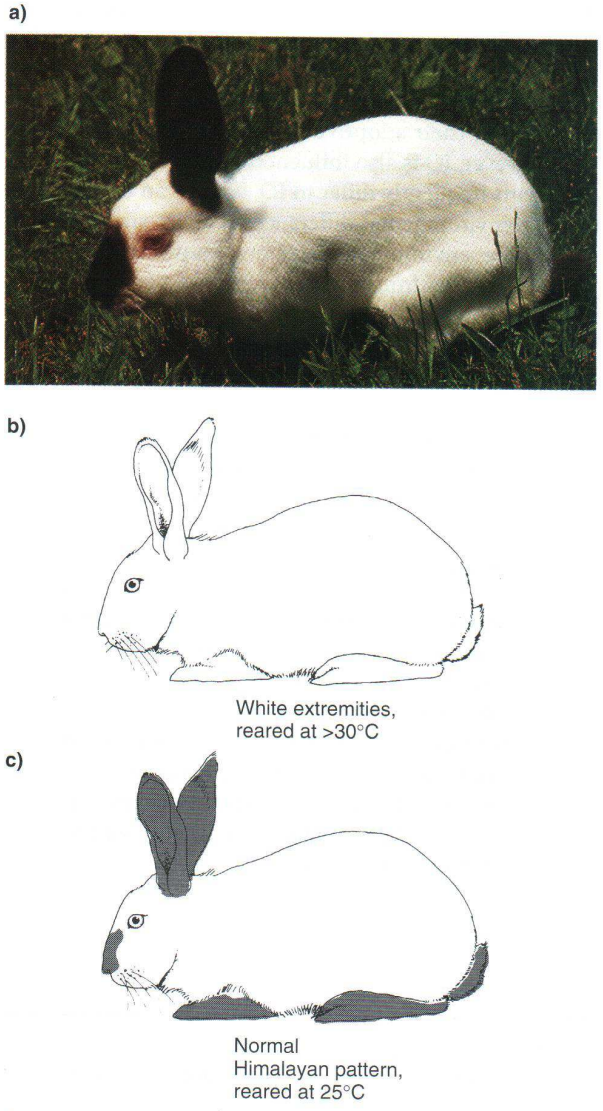


Figure 2.1

Influences on the physical manifestation (phenotype) of the genetic blueprint (genotype): interactions with other genes and their products (such as hormones) and with the environment (such as nutrition).

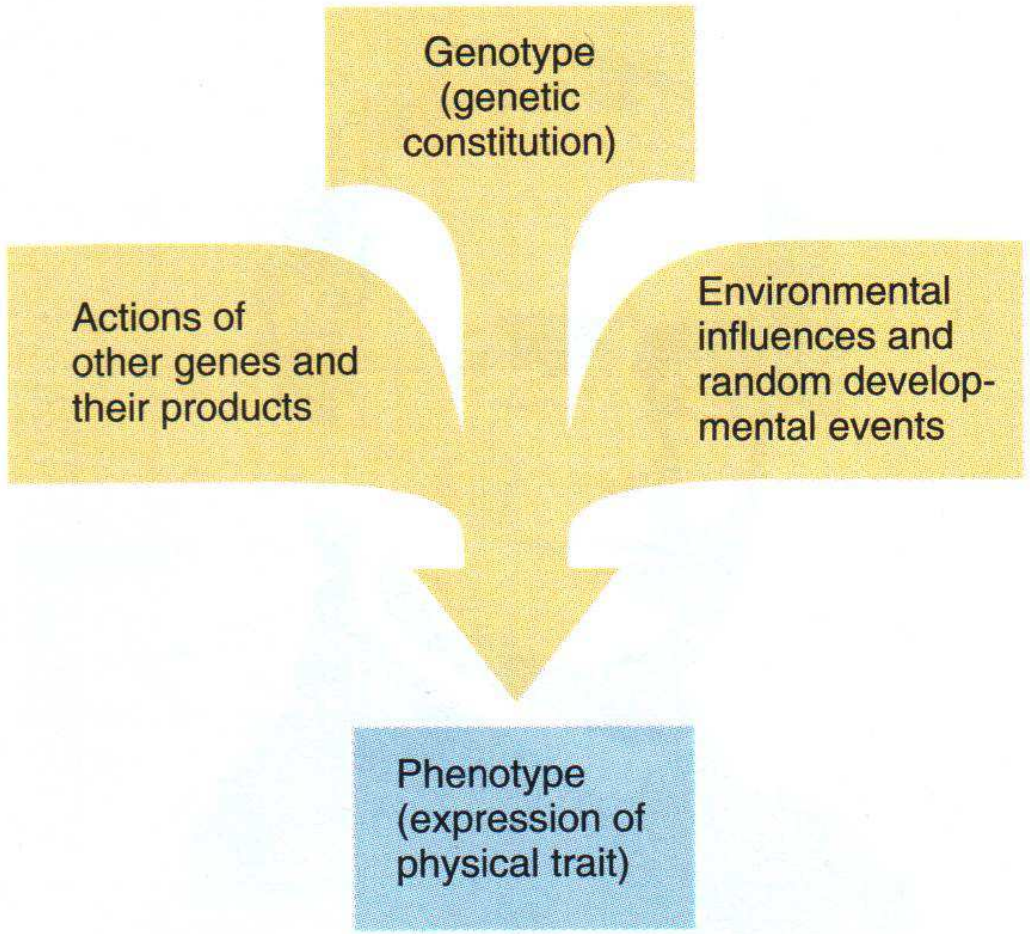
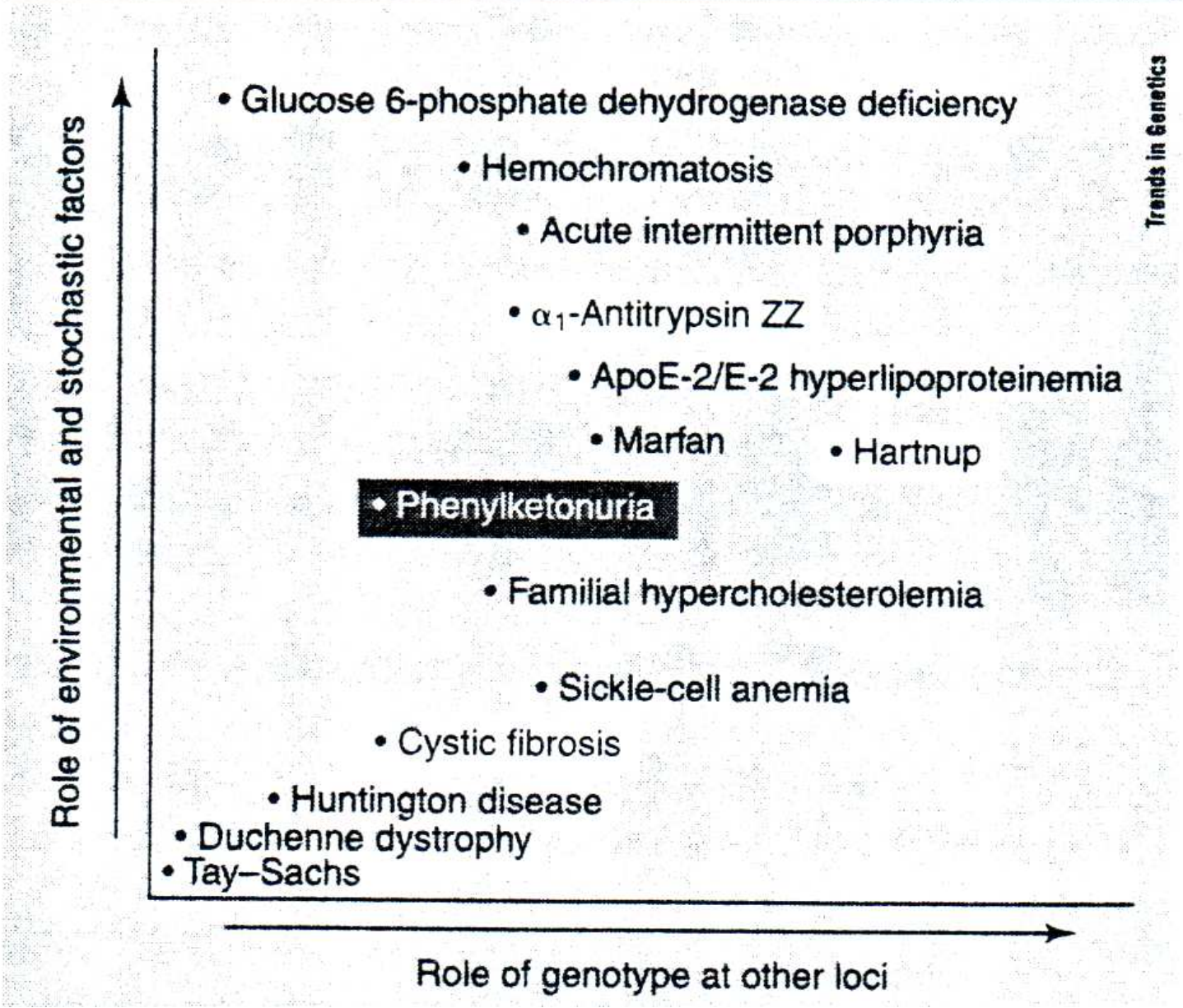
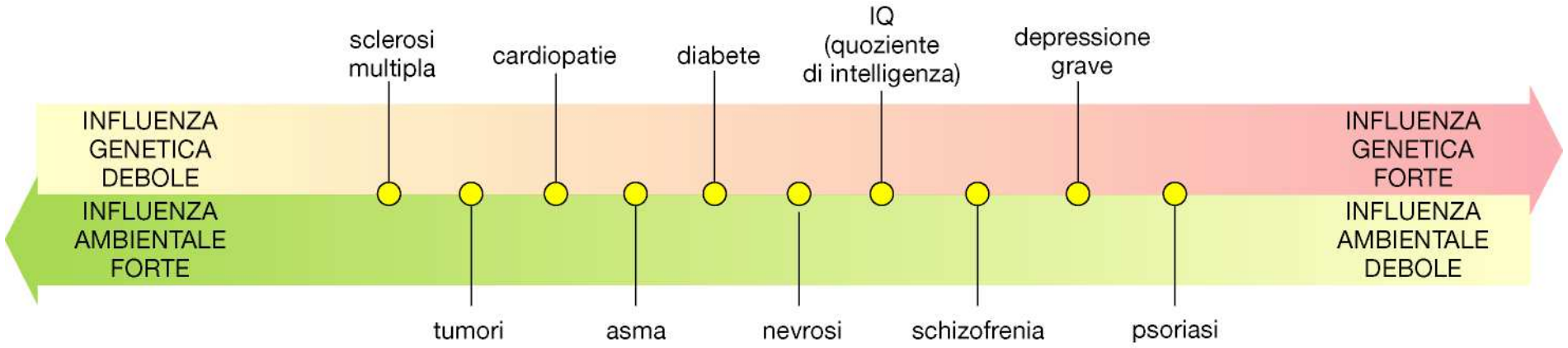


FIGURE 1. Genotype–phenotype relationship




sclerosi multipla
cardiopatie
diabete
IQ (quoziente di intelligenza)
depressione grave
INFLUENZA GENETICA DEBOLE
INFLUENZA AMBIENTALE FORTE
INFLUENZA GENETICA FORTE
INFLUENZA AMBIENTALE DEBOLE
tumori
asma
nevrosi
schizofrenia
psoriasi

La mancata corrispondenza tra genotipo e fenotipo può essere dovuta a:

- Eterogeneità genetica

- Eterogeneità clinica

Mutazioni dinamiche

- Notevoli espansioni nucleotidiche in regioni non codificanti

- Modeste espansioni CAG in regioni codificanti

Anticipazione e **premutazione**

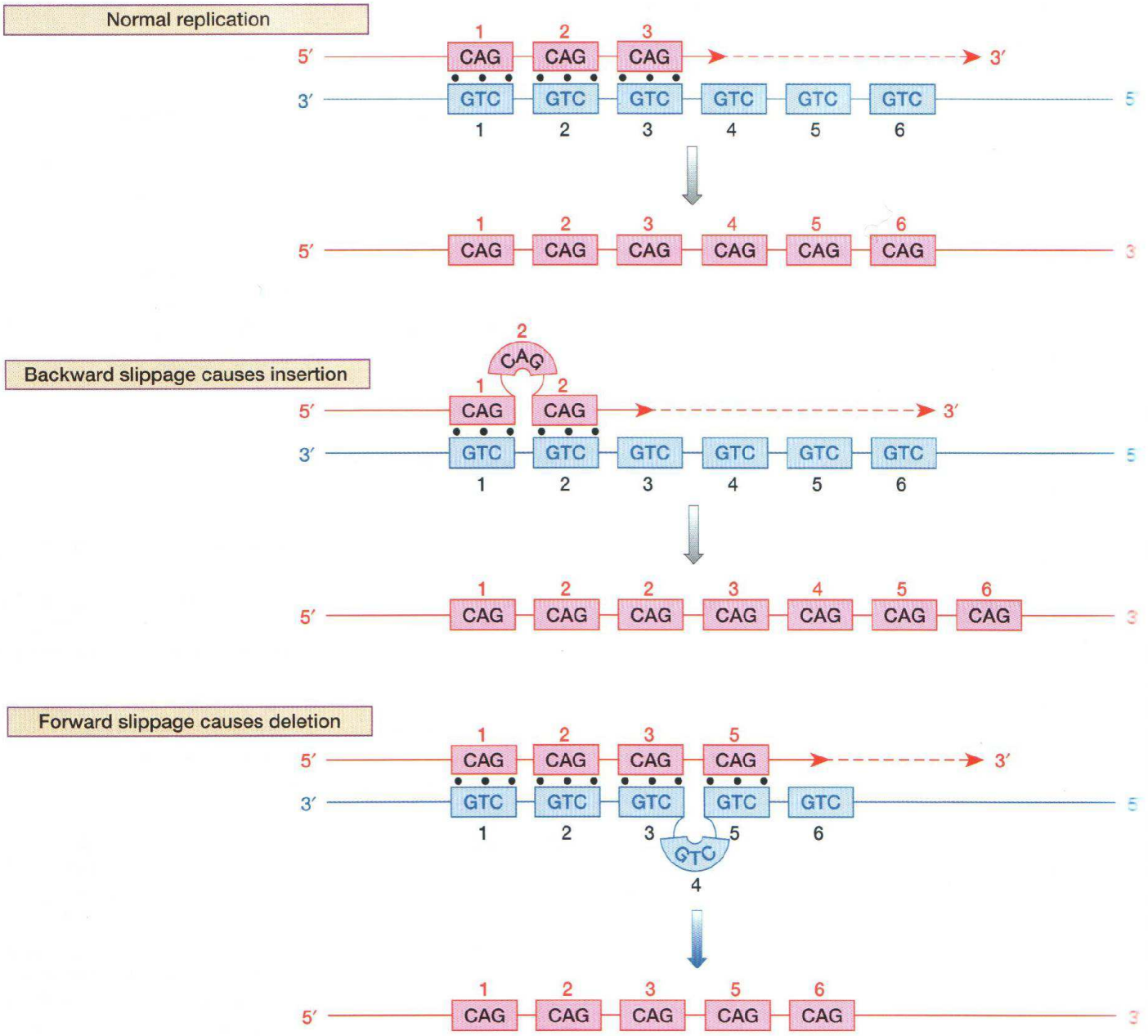
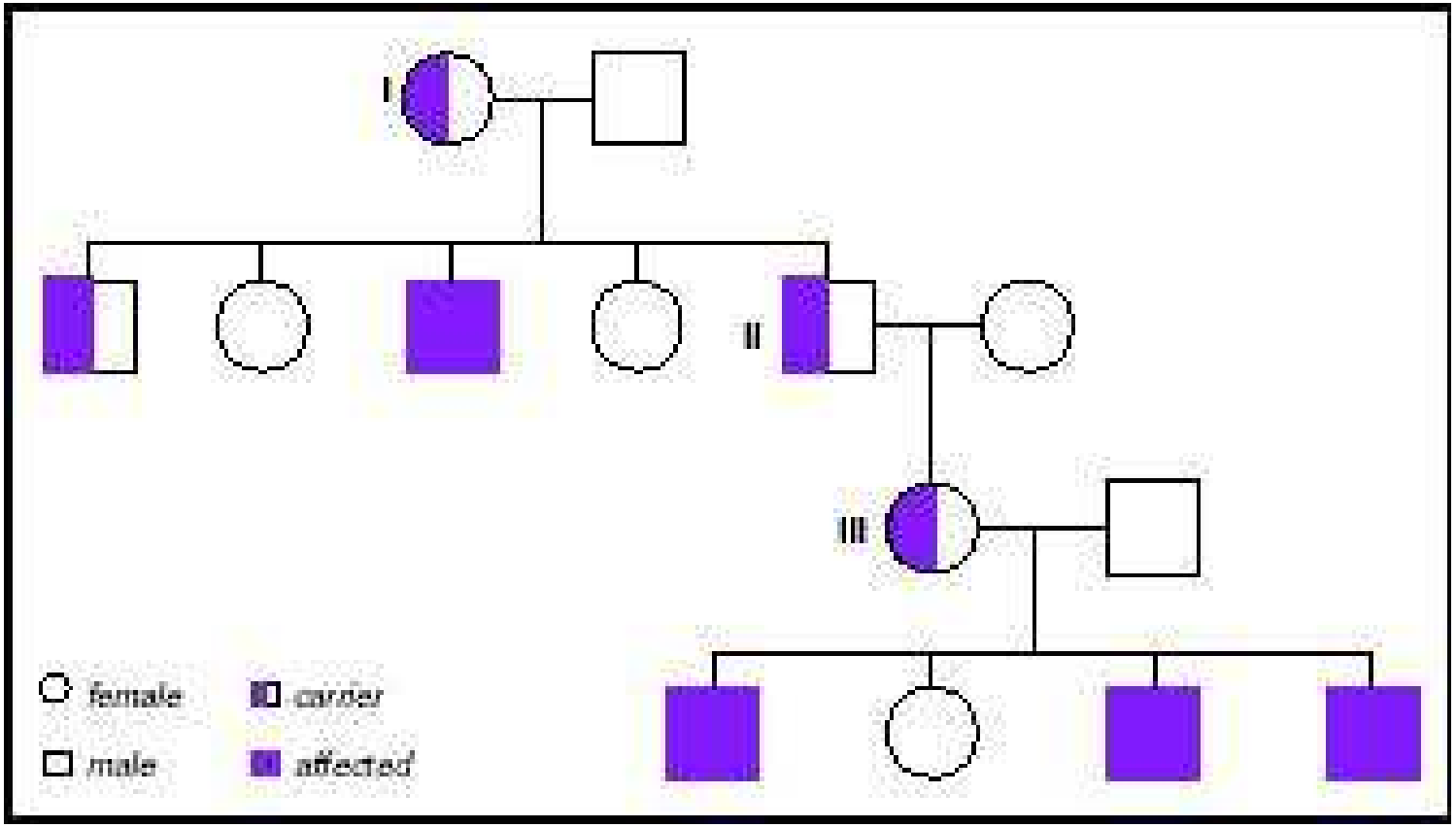

Normal replication
5′
3′
CAG
GTC
Backward slippage causes insertion
Forward slippage causes deletion

Table 16.6: Diseases caused by unstable expanding nucleotide repeats

Disease	MIM No.	Mode of inheritance	Location of gene	Location of repeat	Repeat sequence	Stable repeat #	Unstable repeat #
1. Very large expansions of repeats outside coding sequences:							
Fragile-X site A (FRAXA)	309550	X	Xq27.3	5'UTR	(CGG)n	6–54	200–1000+
Fragile-X site E (FRAXE)	309548	X	Xq28	Promoter	(CCG)n	6–25	200+
Friedreich ataxia (FA)	229300	AR	9q13-q21.1	Intron 1	(GAA)n	7–22	200–1700
Myotonic dystrophy (DM1)	160900	AD	19q13	3'UTR	(CTG)n	5–35	50–4000
Myotonic dystrophy 2 (DM2)	602668	AD	3q21	Intron 1	(CCTG)n	12	75–11 000
Spinocerebellar ataxia 8	603680	AD	13q21	Untranslated RNA	(CTG)n	16–37	110–500+
Spinocerebellar ataxia 10	603516	AD	22q13	Intron 9	(ATTCT)n	10–22	Up to 22 kb
Juvenile myoclonus epilepsy (JME)	254800	AR	21q22.3	Promoter	(CCCCGCCCCGCG)n	2–3	40–80
2. Modest expansions of CAG repeats within coding sequences:							
Huntington disease (HD)	143100	AD	4p16.3	Coding	(CAG)n	6–35	36–100+
Kennedy disease	313200	XR	Xq21	Coding	(CAG)n	9–35	38–62
SCA1	164400	AD	6p23	Coding	(CAG)n	6–38	39–83
SCA2	183090	AD	12q24	Coding	(CAG)n	14–31	32–77
Machado-Joseph disease (SCA3)	109150	AD	14q32.1	Coding	(CAG)n	12–39	62–86
SCA6	183086	AD	19p13	Coding	(CAG)n	4–17	21–30
SCA7	164500	AD	3p12-p21.1	Coding	(CAG)n	7–35	37–200
SCA17	607136	AD	6q27	Coding	(CAG)n	25–42	47–63
Dentatorubral-pallidoluysian atrophy (DRPLA)	125370	AD	12p	Coding	(CAG)n	3–35	49–88

SCA, spino-cerebellar ataxia.



The Sherman Paradox. The daughter (III) of an unaffected male carrier (II) is more likely to have affected offspring than the mother (I) of the male carrier is.

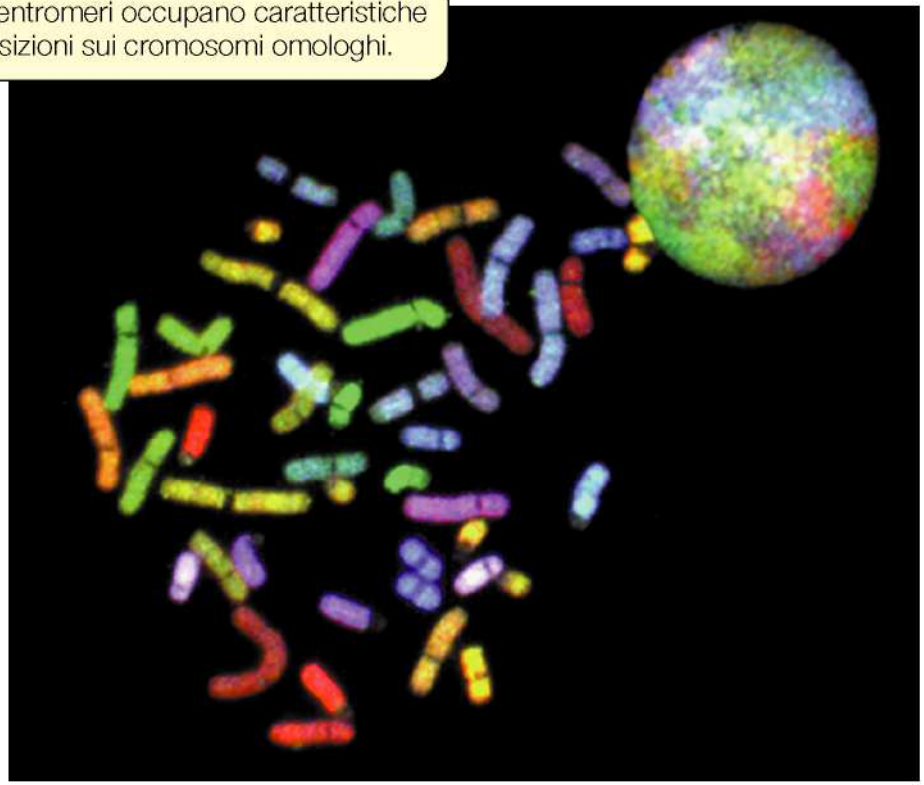
Polimorfismi nel DNA:

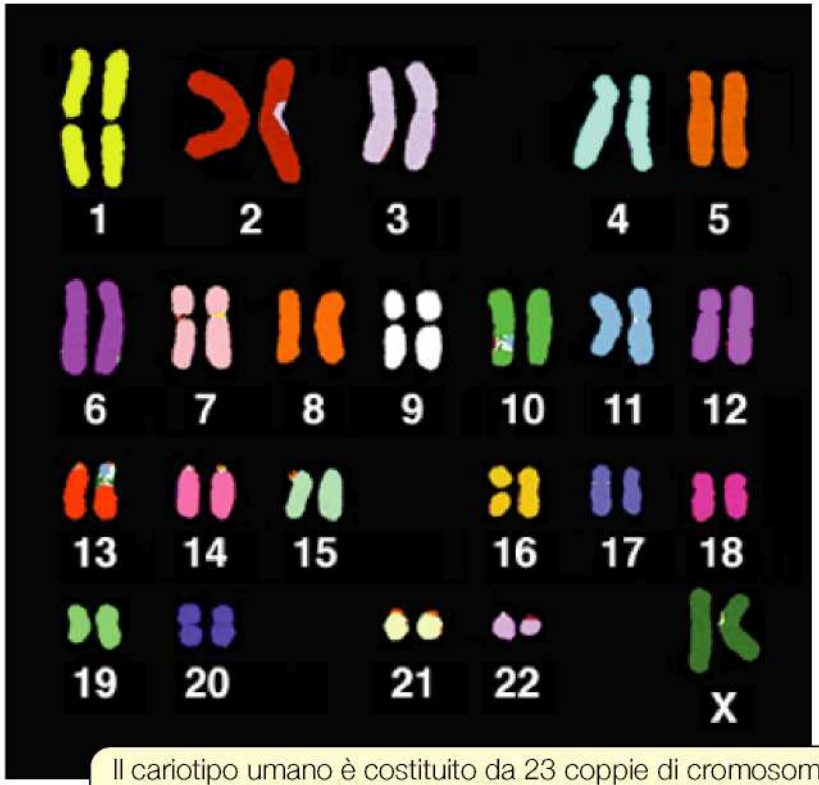
Indicano la presenza di più di una variante allelica, nella popolazione umana, con una frequenza ≥ 1%

Determinazione del sesso

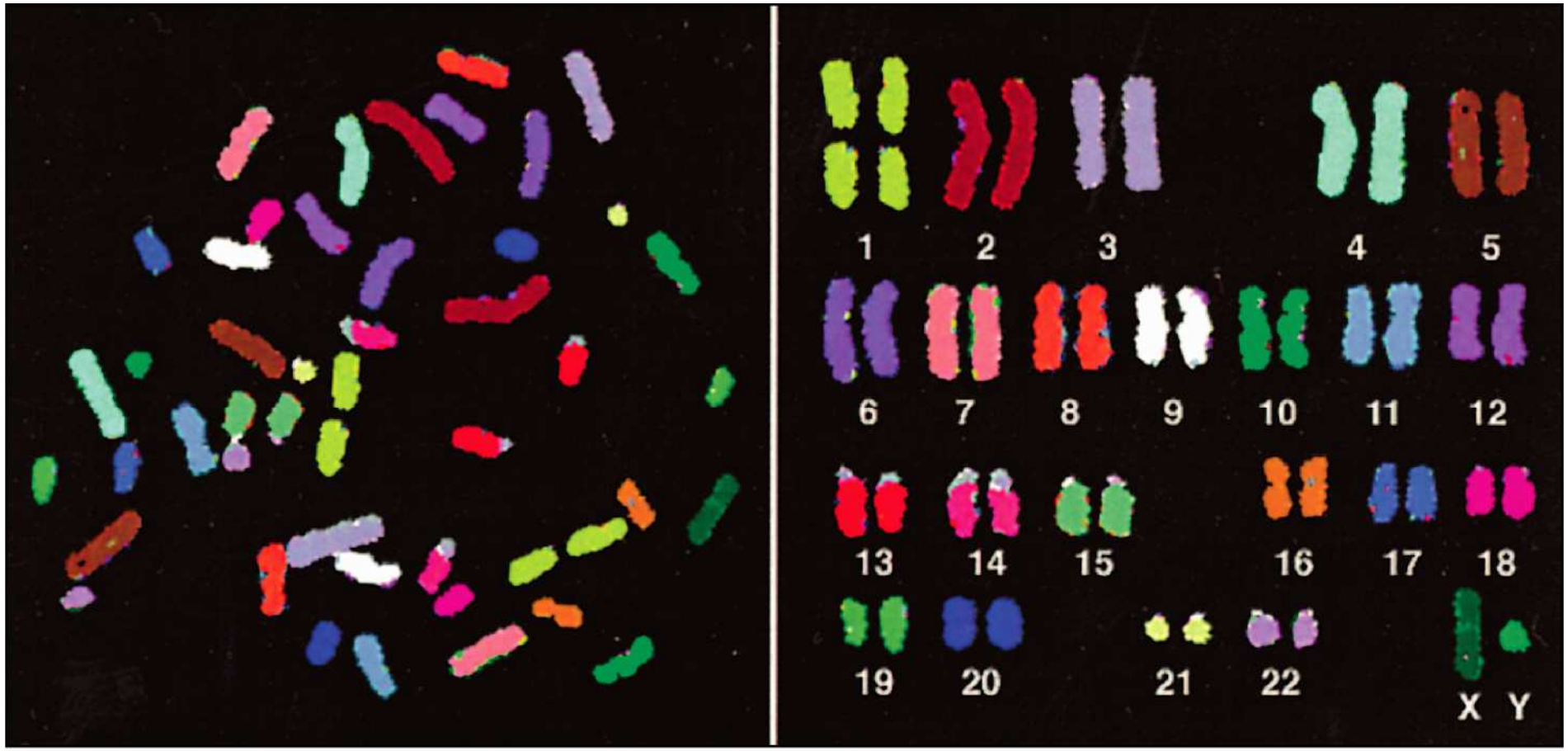
- Sesso cromosomico
- Sesso gonadico
- Sesso fenotipico

I centromeri occupano caratteristiche posizioni sui cromosomi omologhi.

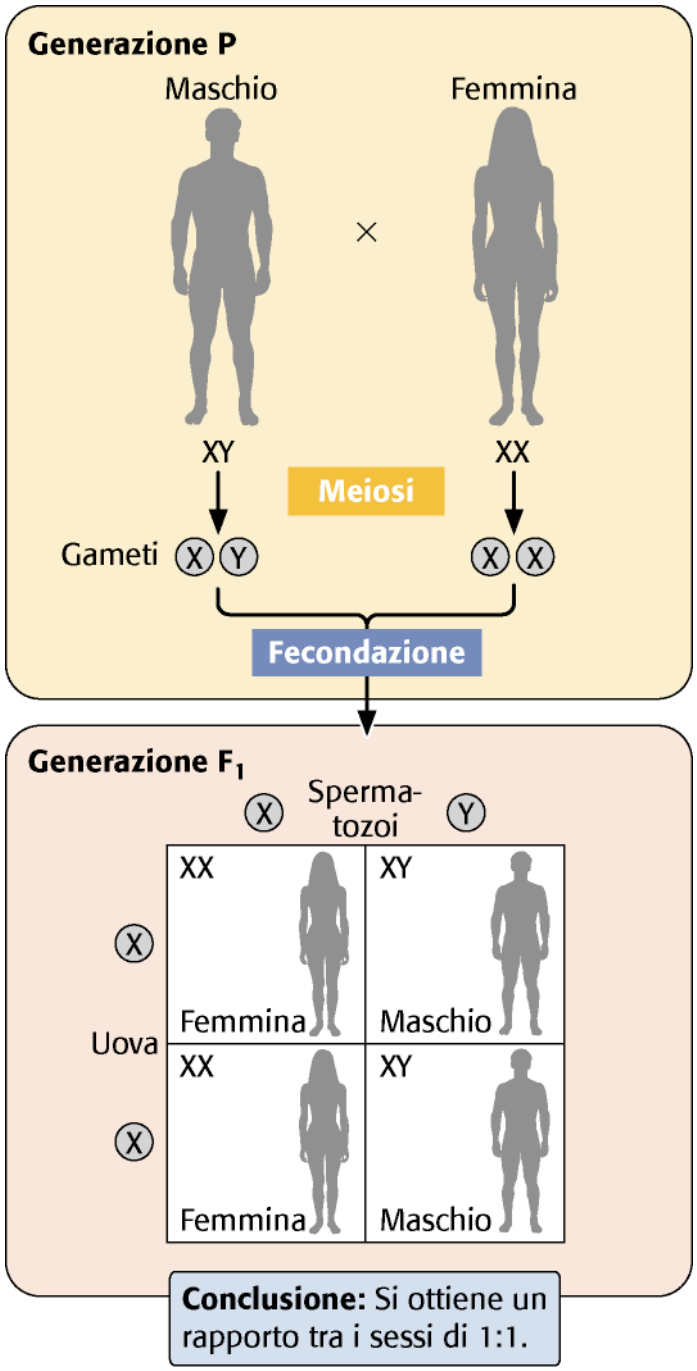


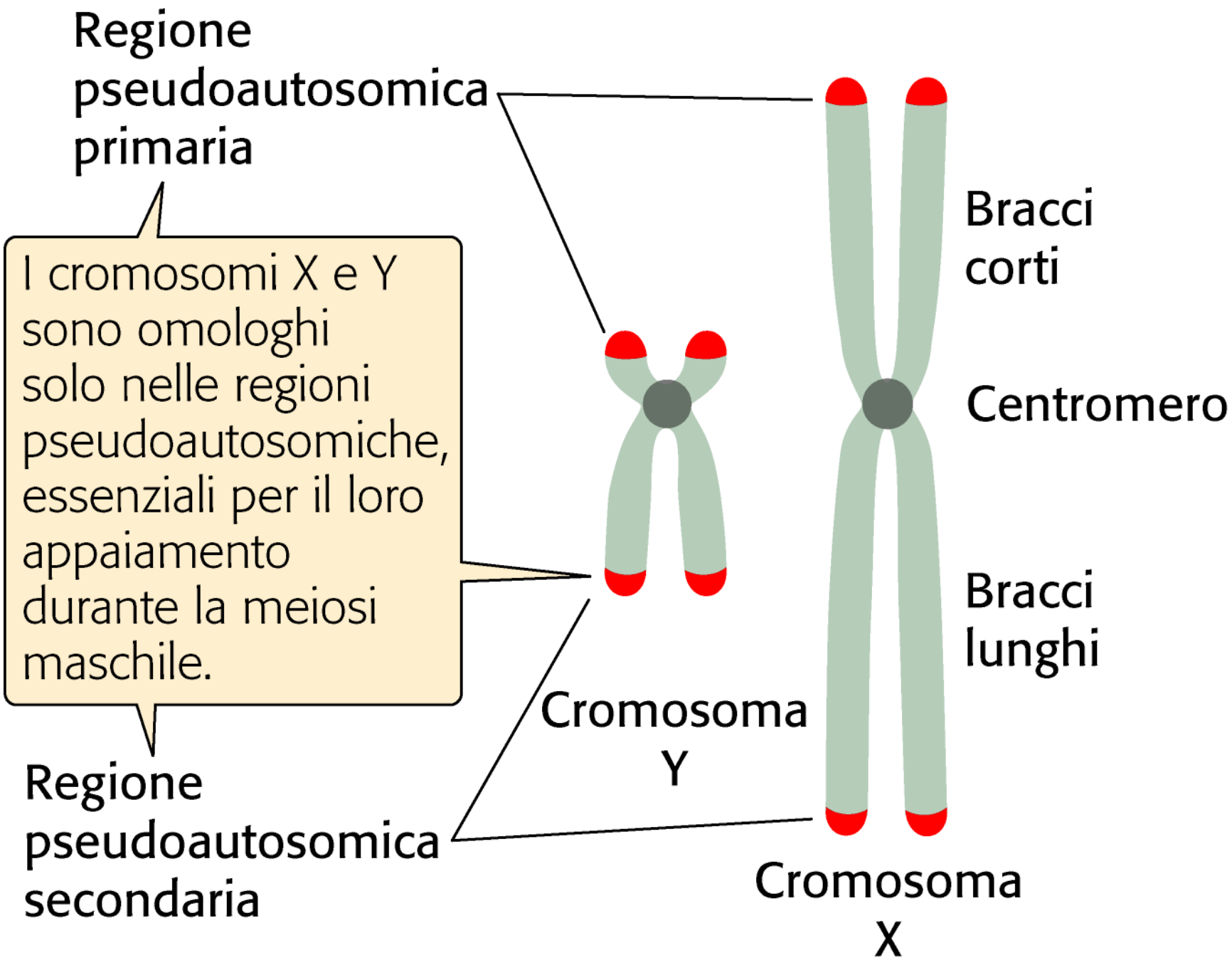


Il cariotipo umano è costituito da 23 coppie di cromosomi, compresi i cromosomi sessuali. I cromosomi sessuali della femmina sono X e X; il maschio ha un cromosoma X e uno Y.



(A) (B)


Generazione P
Maschio
Femmina
×
XY
XX
Meiosi
Gameti
X
Y
X
X
Fecondazione
Generazione F1
X
Sperma-
tozoi
Y
XX
XY
X
Femmina
Maschio
Uova
XX
XY
X
Femmina
Maschio
Conclusione: Si ottiene un rapporto tra i sessi di 1:1.


Regione pseudoautosomica primaria
I cromosomi X e Y sono omologhi solo nelle regioni pseudoautosomiche, essenziali per il loro appaiamento durante la meiosi maschile.
Regione pseudoautosomica secondaria
Cromosoma Y
Bracci corti
Centromero
Bracci lunghi
Cromosoma X

Nell’uomo la determinazione del sesso è stabilita alla fertilizzazione e avviene su base cromosomica.

La differenziazione sessuale invece comincia intorno alla quinta settimana di vita embrionale. Nella differenziazione sessuale dobbiamo distinguere:

- Caratteristiche sessuali primarie (cariotipo)

- Caratteristiche sessuali secondarie (ormoni)

Nella determinazione genotipica del sesso i cromosomi sessuali giocano un ruolo chiave nella ereditarietà e nella determinazione del sesso.

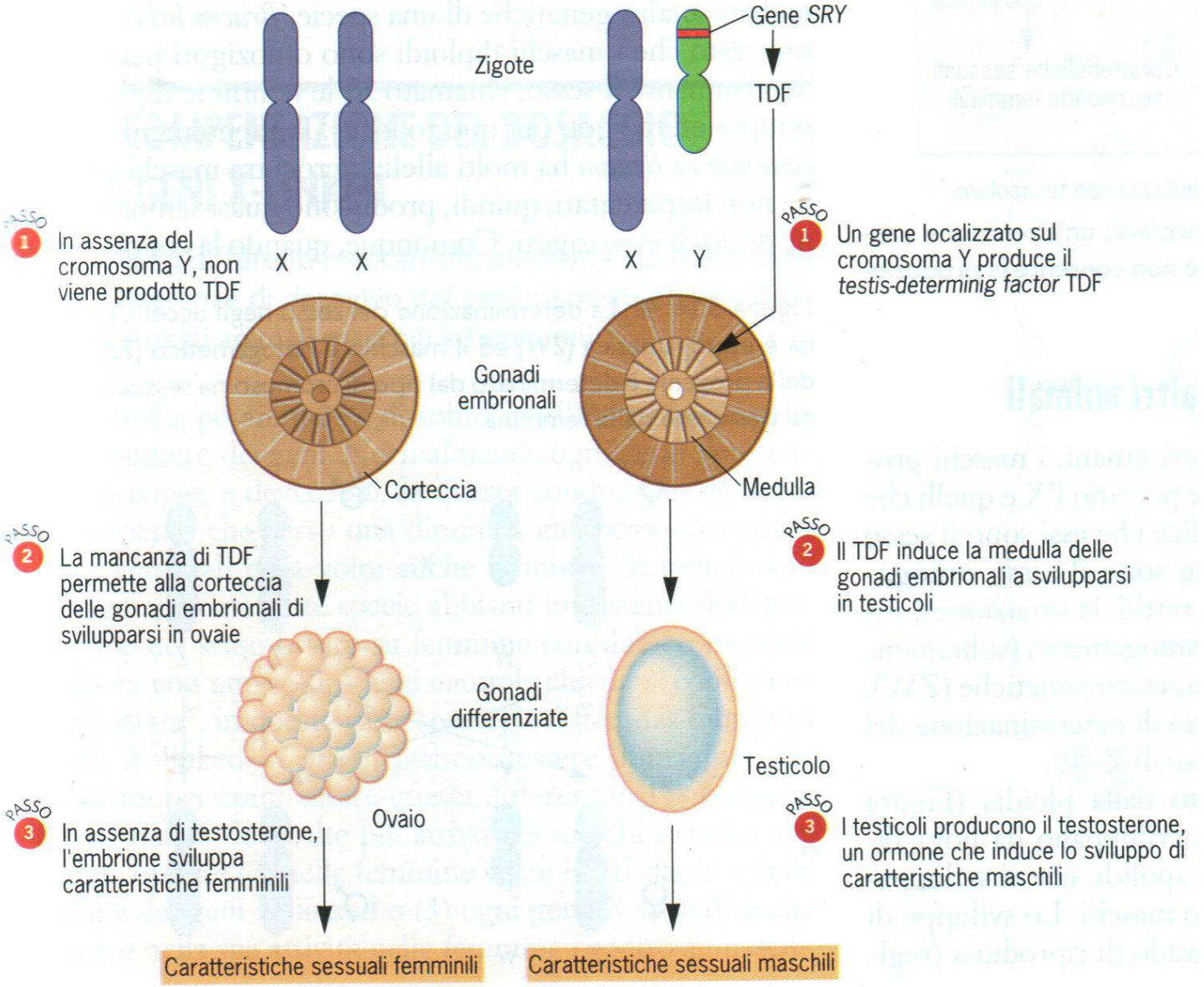
- Sesso determinato dalla presenza, o meno, del cromosoma Y

- Sesso determinato dal rapporto tra il numero di cromosomi X e il numero di set di autosomi

Table 3.2 **Consequences of Various X and Y Chromosome Abnormalities in Humans, Showing Role of the Y in Sex Determination**

Chromosome Constitution[a]	Designation of Individual	Expected Number of Barr Bodies
46,XX	Normal ♀	1
46,XY	Normal ♂	0
45,X	Turner syndrome ♀	0
47,XXX	Triplo-X ♀	2
47,XXY	Klinefelter syndrome ♂	1
48,XXXY	Klinefelter syndrome ♂	2
48,XXYY	Klinefelter syndrome ♂	1
47,XYY	XYY syndrome ♂	0

[a]The first number indicates the total number of chromosomes in the nucleus, and the Xs and Ys indicate the sex chromosome complement.


Zigote
Gene SRY
TDF
X
X
X
Y
PASSO 1
In assenza del cromosoma Y, non viene prodotto TDF
PASSO 1
Un gene localizzato sul cromosoma Y produce il *testis-determinig factor* TDF
Gonadi embrionali
Corteccia
Medulla
PASSO 2
La mancanza di TDF permette alla corteccia delle gonadi embrionali di svilupparsi in ovaie
PASSO 2
Il TDF induce la medulla delle gonadi embrionali a svilupparsi in testicoli
Gonadi differenziate
Ovaio
Testicolo
PASSO 3
In assenza di testosterone, l'embrione sviluppa caratteristiche femminili
PASSO 3
I testicoli producono il testosterone, un ormone che induce lo sviluppo di caratteristiche maschili
Caratteristiche sessuali femminili
Caratteristiche sessuali maschili

Maschi XX hanno un frammento del cromosoma Y (estremità del braccio corto) traslocato su un cromosoma X.

Femmine XY presentano una delezione che interessa l’estremità del braccio corto del cromosoma Y.

Il cromosoma Y contiene il locus per il **testis-determining factor**.

SRY.

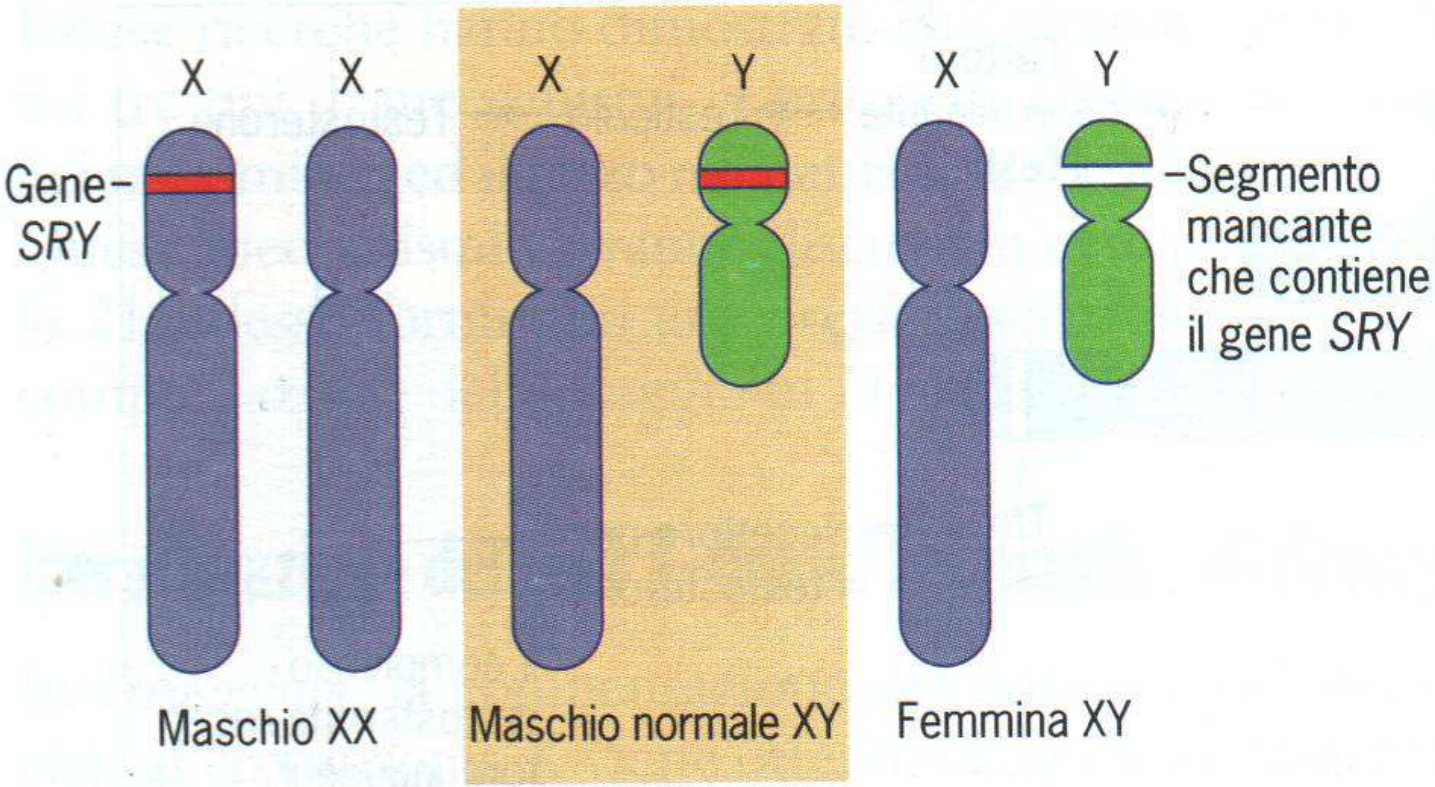


Figura 5.12 ■ Prove che localizzano il gene per il fattore TDF sul braccio corto del cromosoma Y in maschi normali. Il TDF è il prodotto del gene *SRY*. In questi maschi XX, una piccola regione che contiene questo gene è traslocata in uno dei cromosomi X; nelle femmine XY, invece, risulta deleta dal cromosoma Y.