Bari, 27 Febbraio 2010
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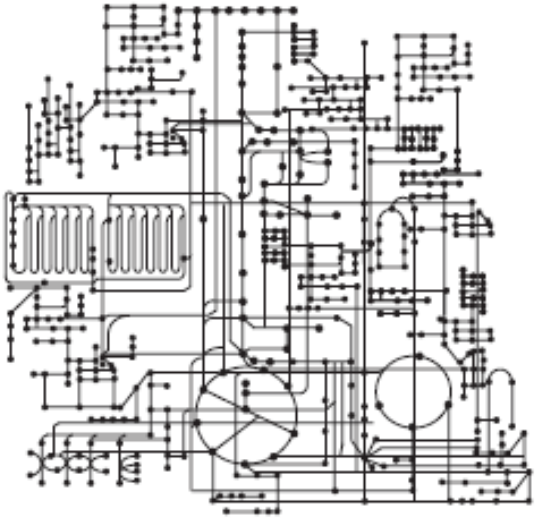
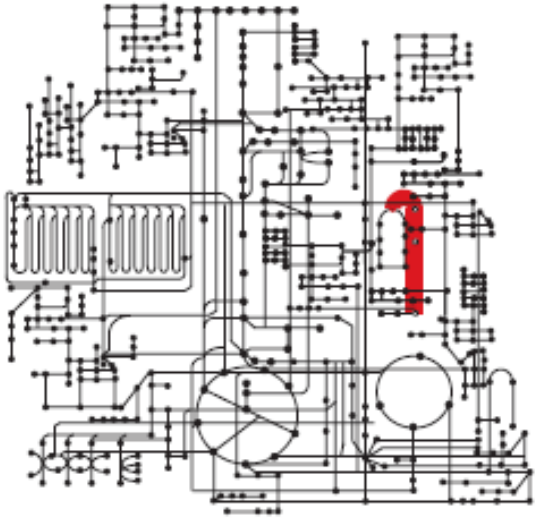
Dipartimento di Biomedicina dell'Età Evolutiva
UOC Lab. Genetica Medica

" INDAGINI GENETICHE: QUANDO E PERCHE' "

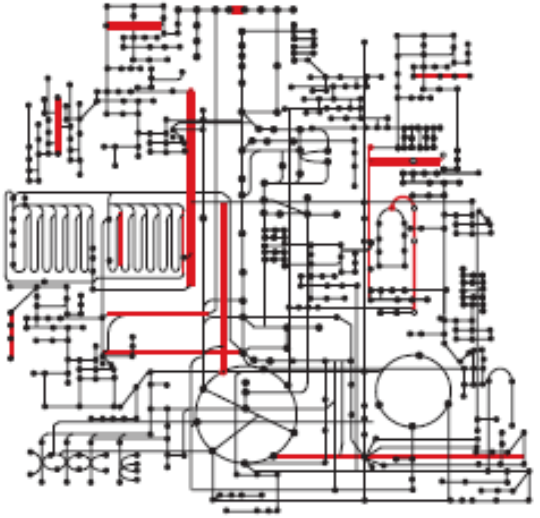
Wild type



Mendelian disorder



Complex disease



EREDITA' MENDELIANA CLASSICA

Tabella 5.1 Ereditarietà mendeliana nell'uomo

Loci	Autosomici	X-Linked	Y-Linked	Mitocondriali	Totali
Geni a sequenza nota	9.934	457	48	37	10.477
Fenotipo con gene a sequenza nota	2.016	177	2	26	2.221
Fenotipi mendeliani senza gene noto	1.361	135	4	0	1.500
Fenotipi potenzialmente mendeliani	2.092	147	2	0	2.241
Totali	**15.403**	**917**	**56**	**63**	**16.439**

Neri G, Genuardi M. Genetica umana e medica. Elsevier Masson, Milano, 2007

~ 600
E C M

1 affetto ogni 200-250 nati

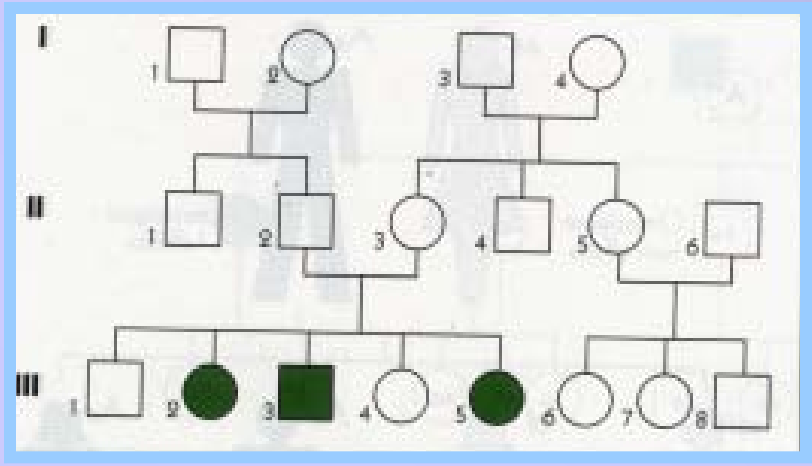
- EREDITA' AUTOSOMICA RECESSIVA
- X-LINKED
- EREDITA' AUTOSOMICA DOMINANTE
- EREDITA' MATRILINEARE O MITOCONDRIALE

10.000
Enzimi

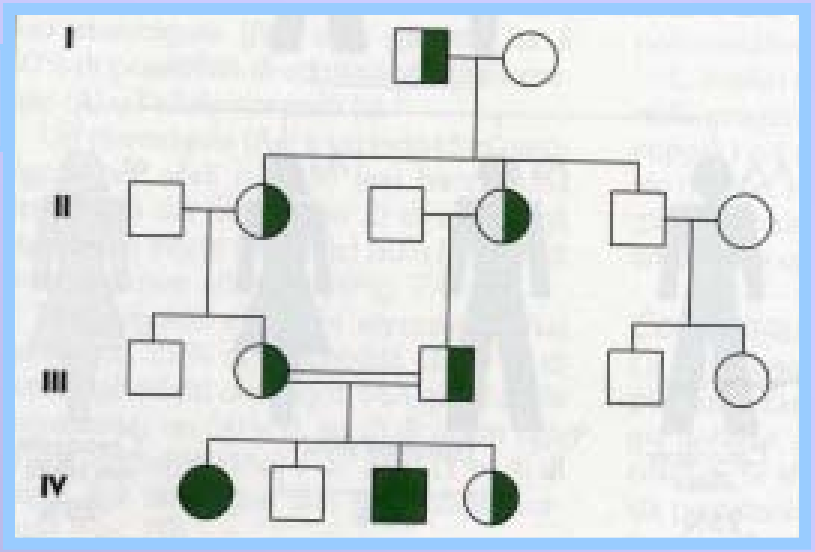
EREDITA' AUTOSOMICA RECESSIVA

Genitori sani

Orizzontale



Consanguineità !



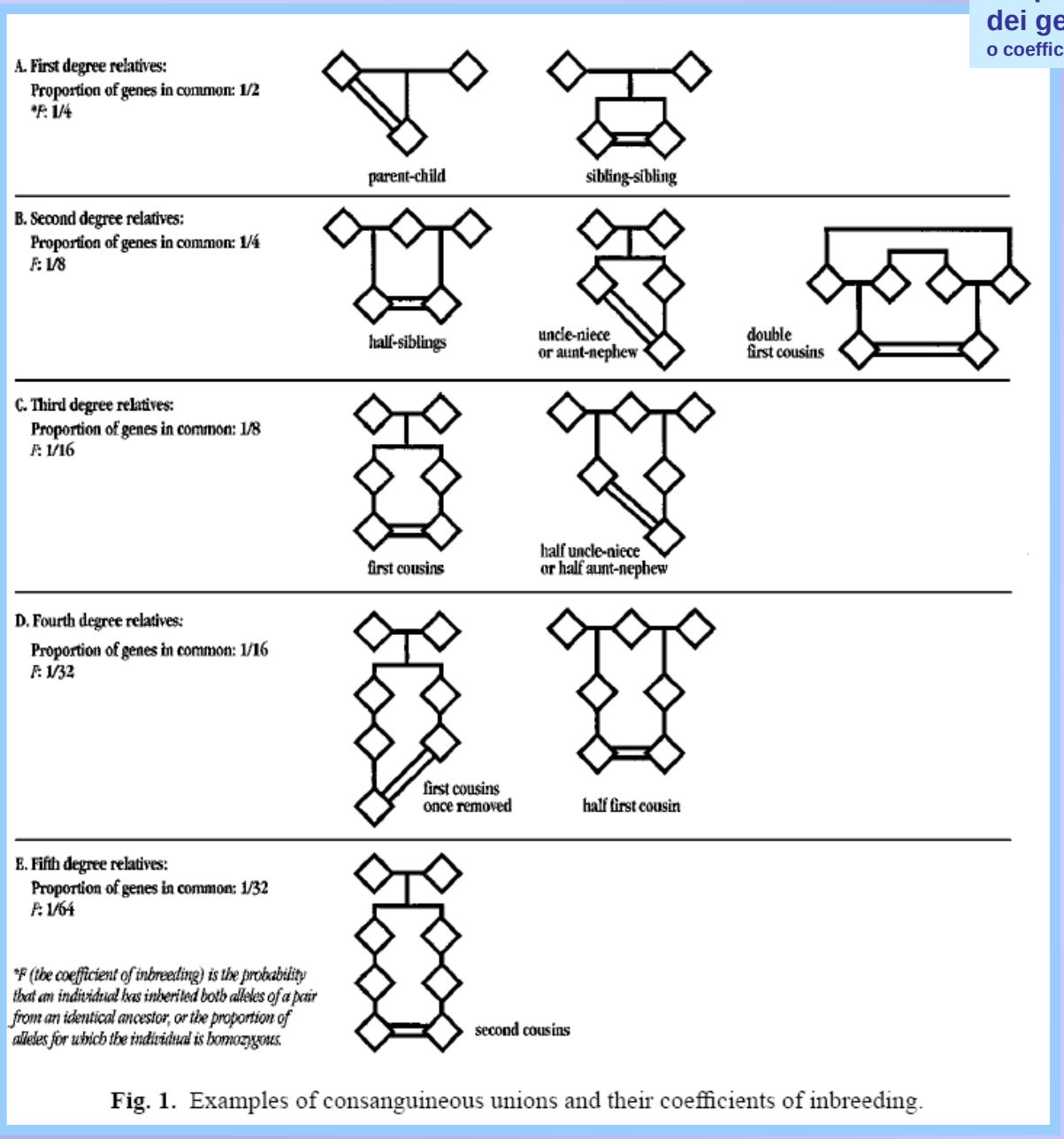
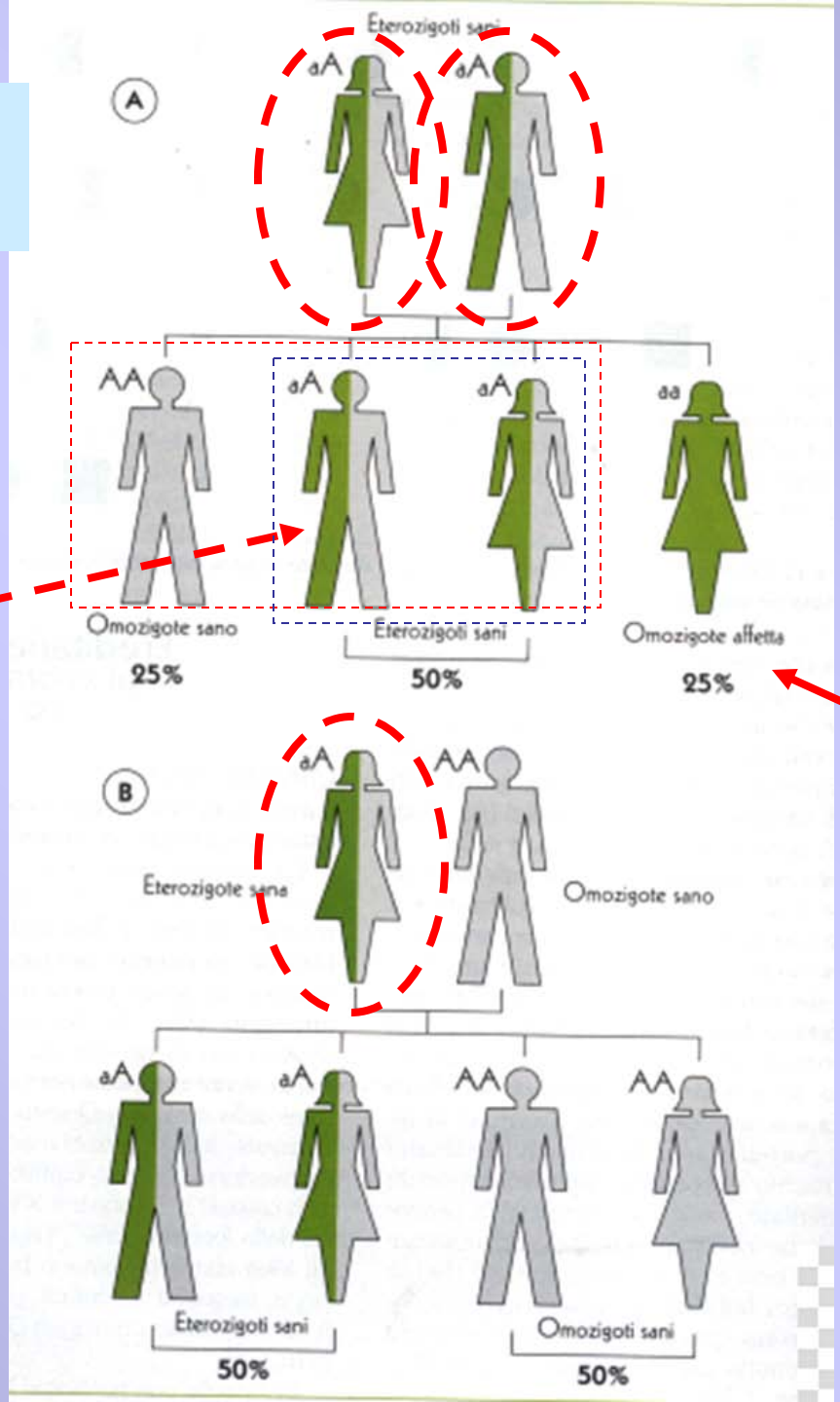


Fig. 1. Examples of consanguineous unions and their coefficients of inbreeding.

Proporzione dei geni in comune o coefficiente di parentela	**Probabilità di omozigosi per discesa** *Coefficiente di consanguineità*
1/2	1/4
1/4	1/8
1/8	1/16
1/16	1/32
1/32	1/64

Rischio Riproduttivo In caso di:

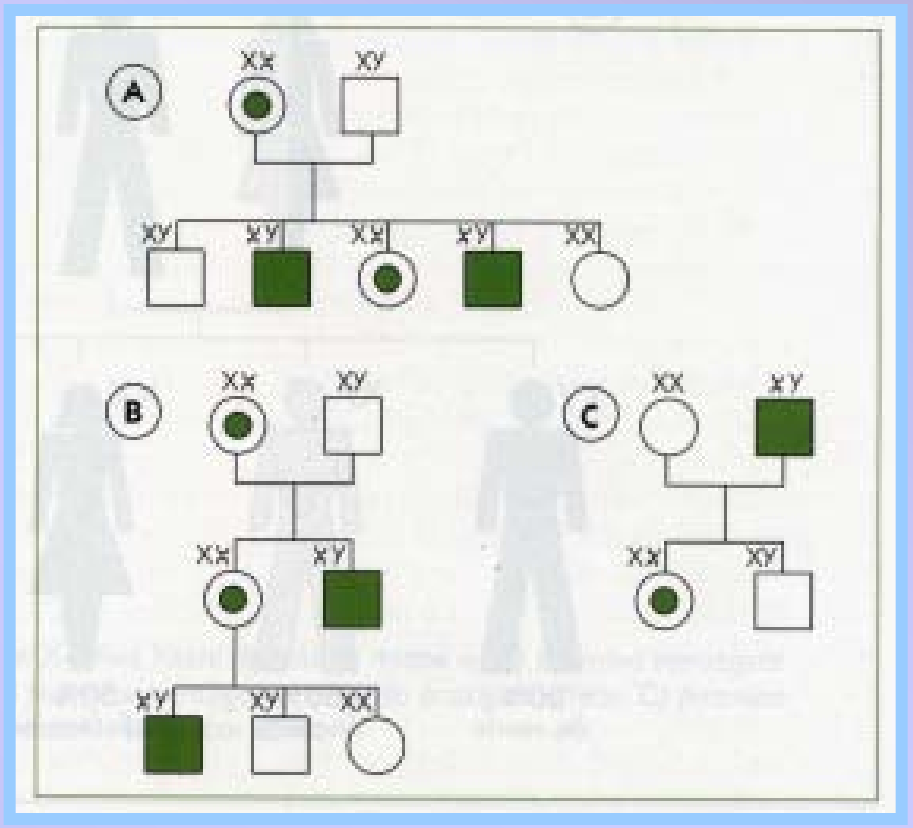
2/3



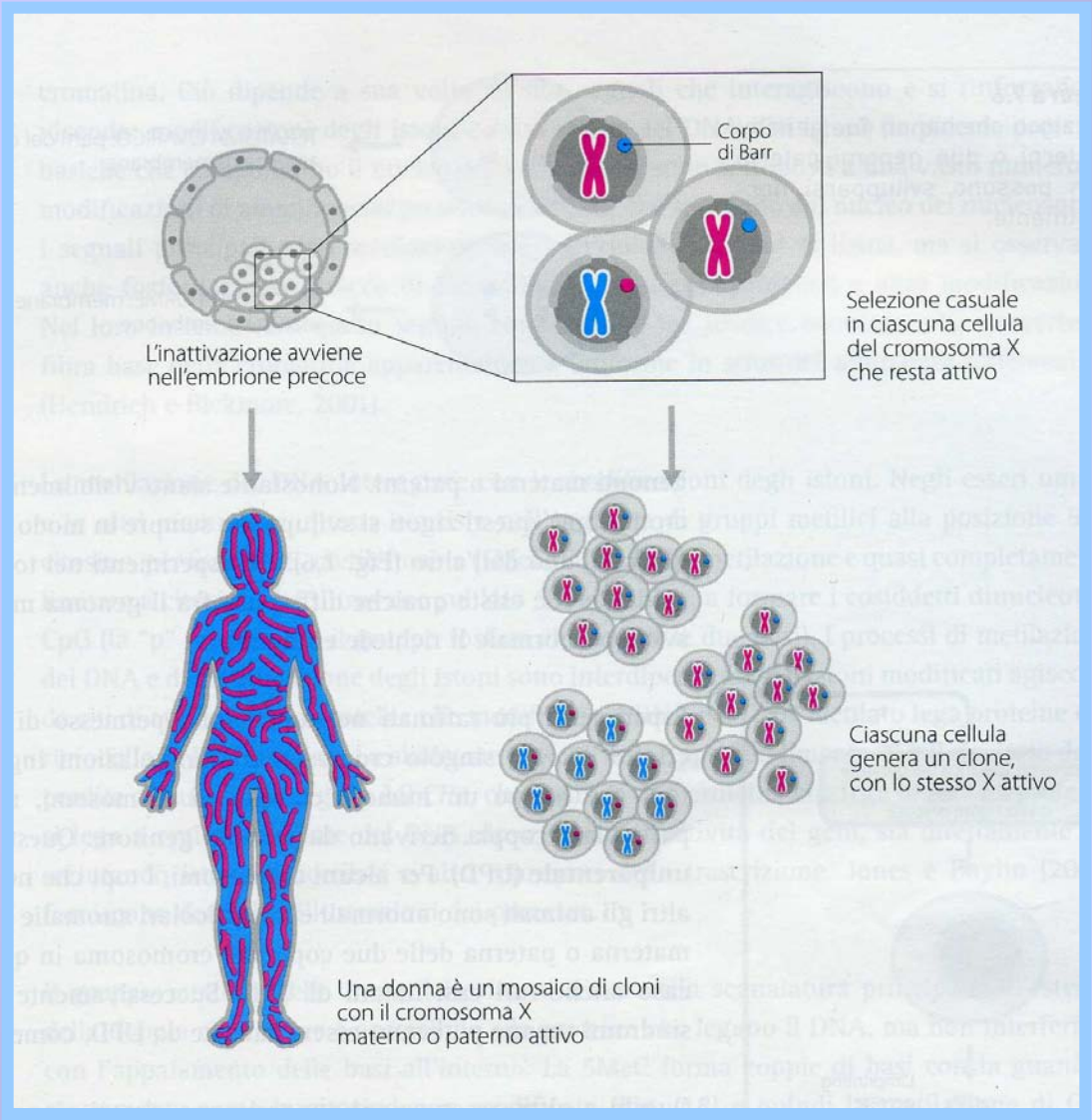
EREDITA’
X-LINKED recessiva

Sono portatrici obbligate le donne:

- **con più di un figlio maschio affetto**
- **con un fratello ed un figlio maschio affetto**
- **figlie di un maschio affetto emizigote (con paternità certa)**

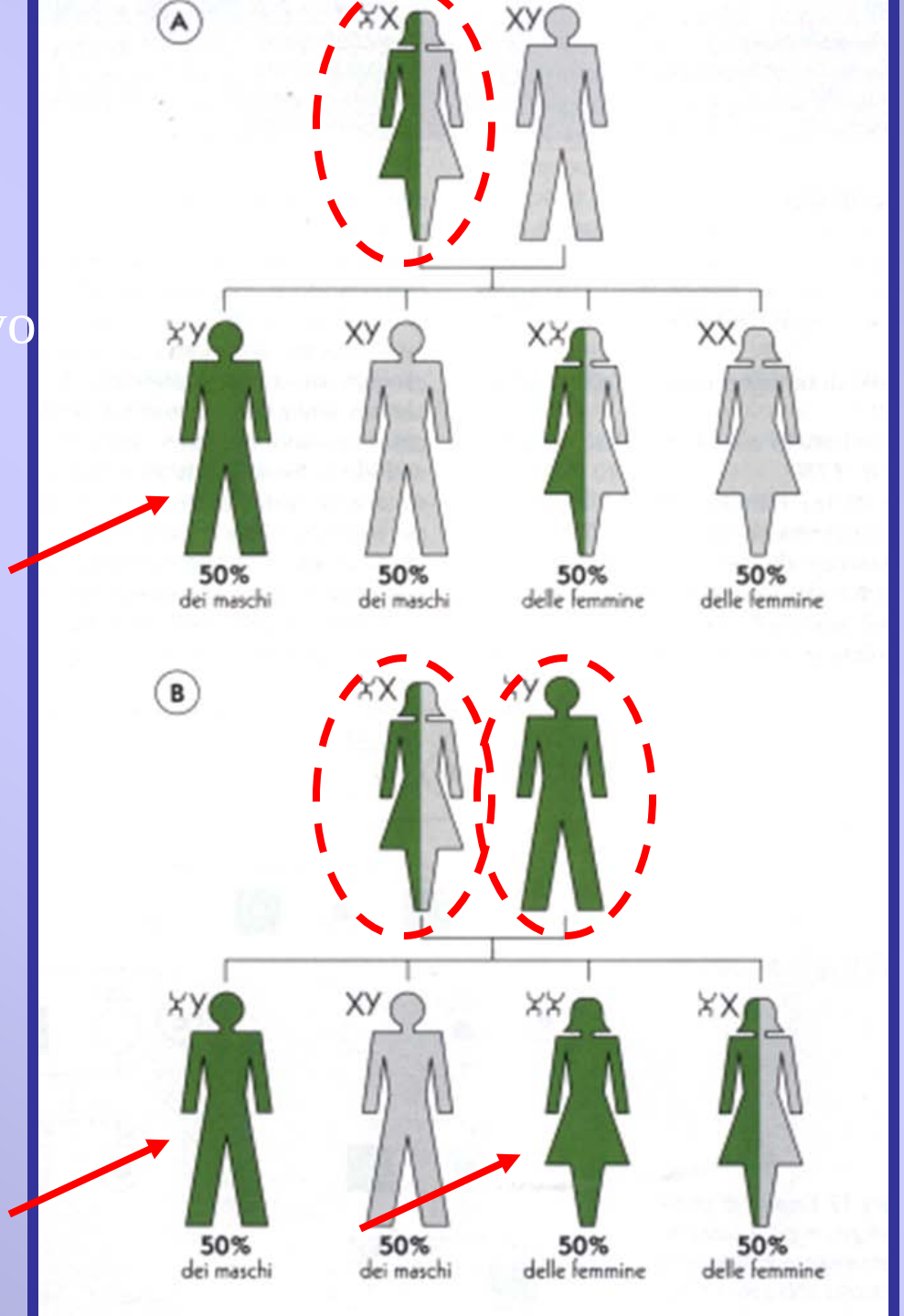


Inattivazione dell'X



M. X-linked Recessive

Rischio Riproduttivo
In caso di:



- ✓ **Eterogeneità allelica**

- ✓ **Impossibilità di evidenziare mutazioni**

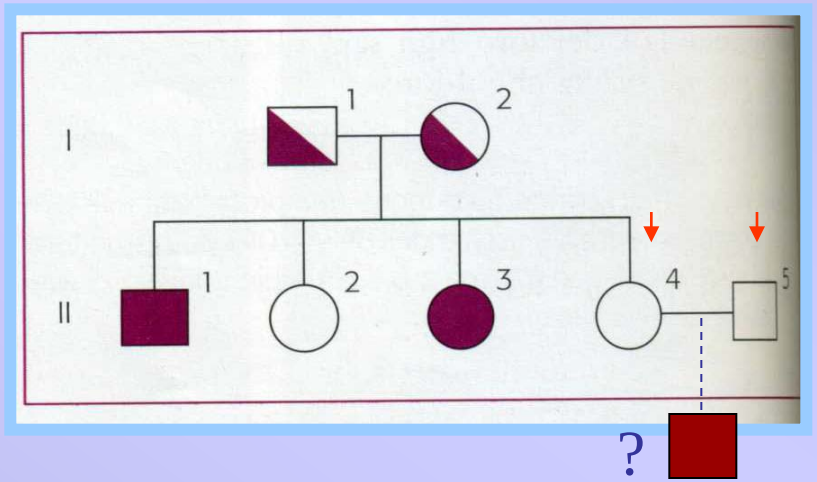
⇩

Calcolo del rischio

⇩

Analisi indiretta utilizzando marcatori del DNA vicini al gene

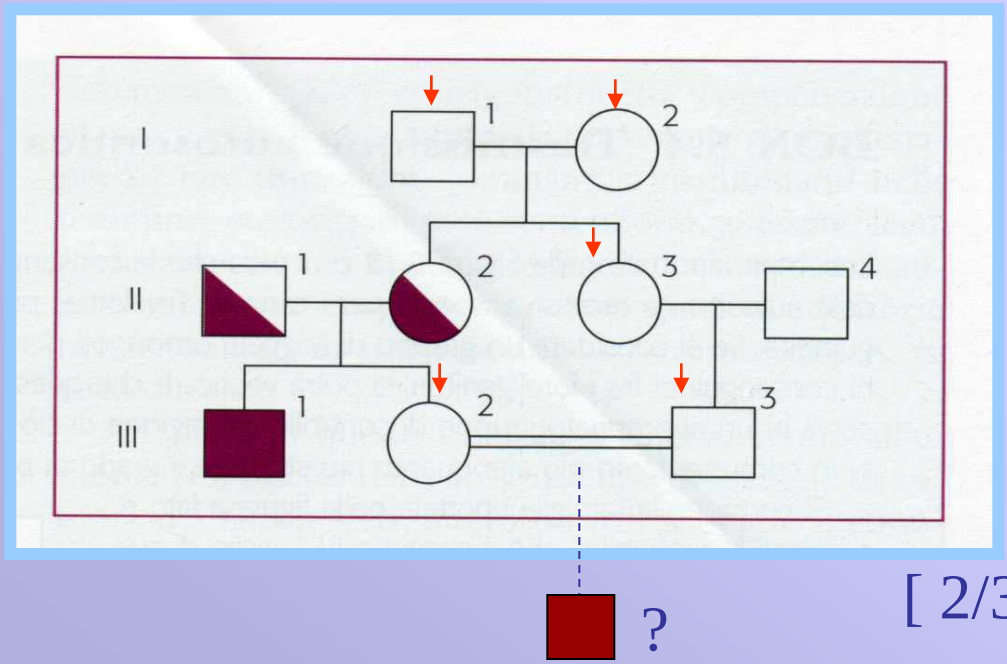
Calcolo del rischio Aut. Recessiva



Rischio di avere un figlio affetto …**0,33** %

[2/3 x Freq.eterozigoti nella popolazione x ¼]

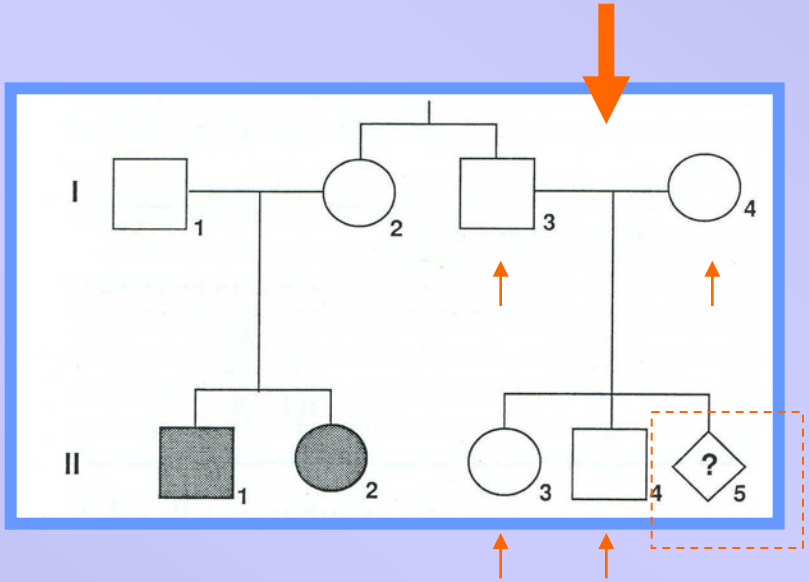
[1/50]



Rischio di avere un figlio affetto …**4 %**

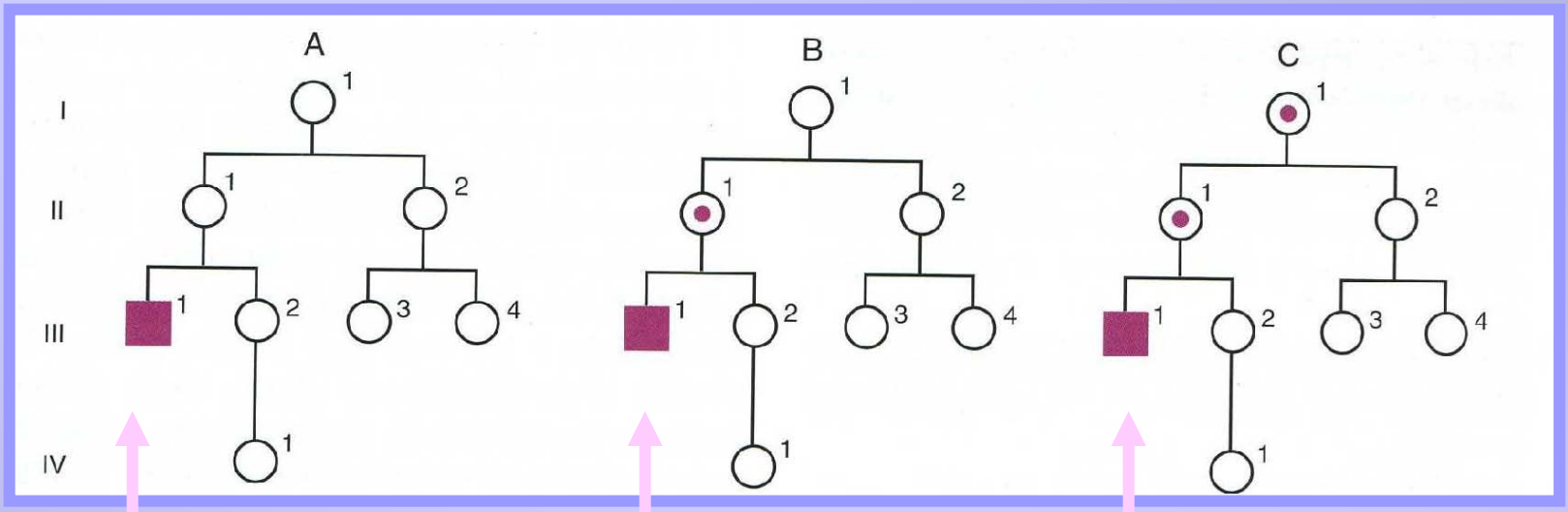
[2/3 x 1/4 x 1/4]

Calcolo del rischio
A R



1/400 → **1/708**

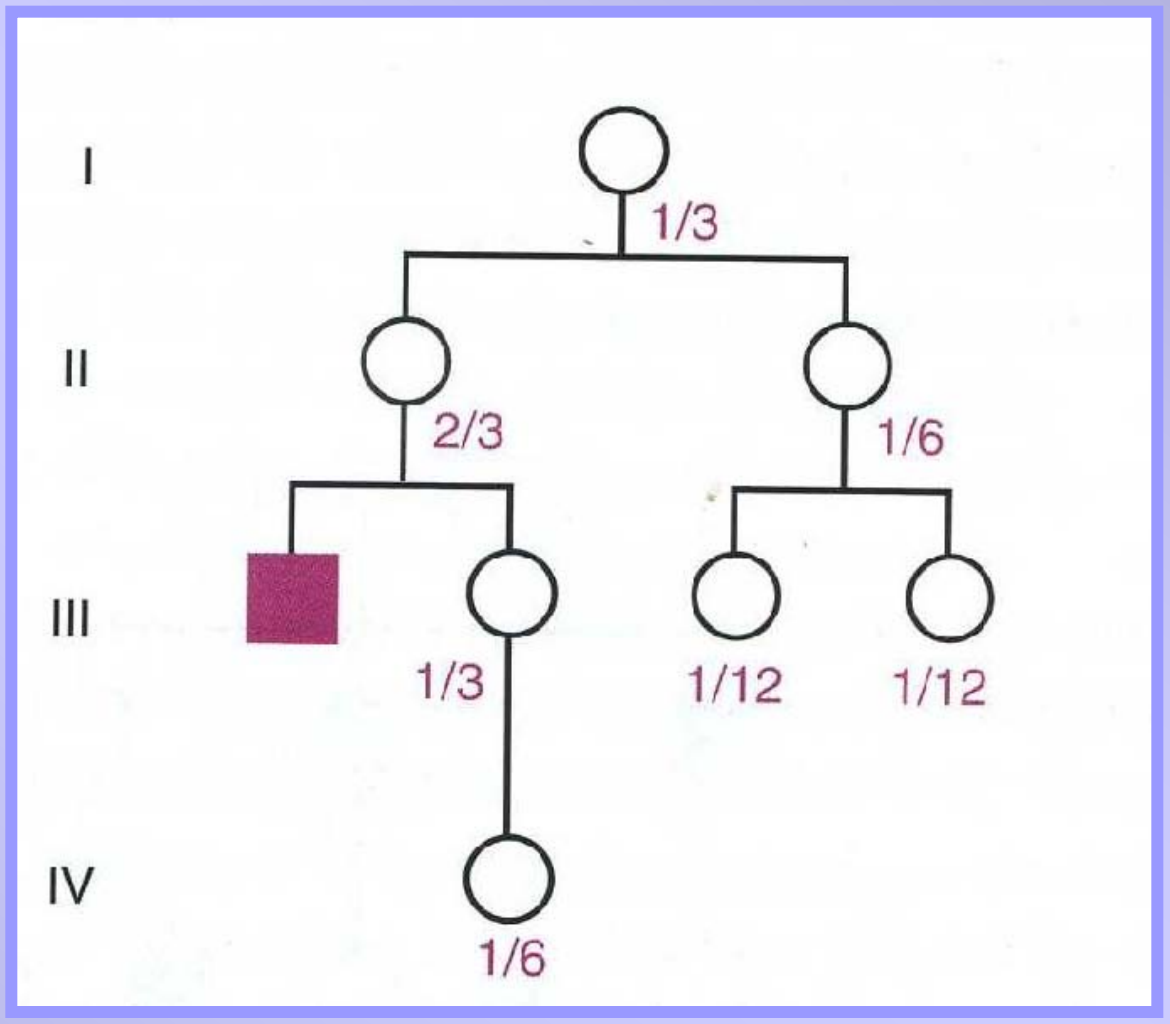
Calcolo del rischio
X- linked



Neomutazione

Madre portatrice di una neomutazione

Sia la madre che la nonna Sono portatrici



Rischio di essere portatrice per tutte le donne

Stima del rischio suscettibile di modificazioni

- ✓ **Eterogeneità allelica**

- ✓ **Impossibilità di evidenziare mutazioni**

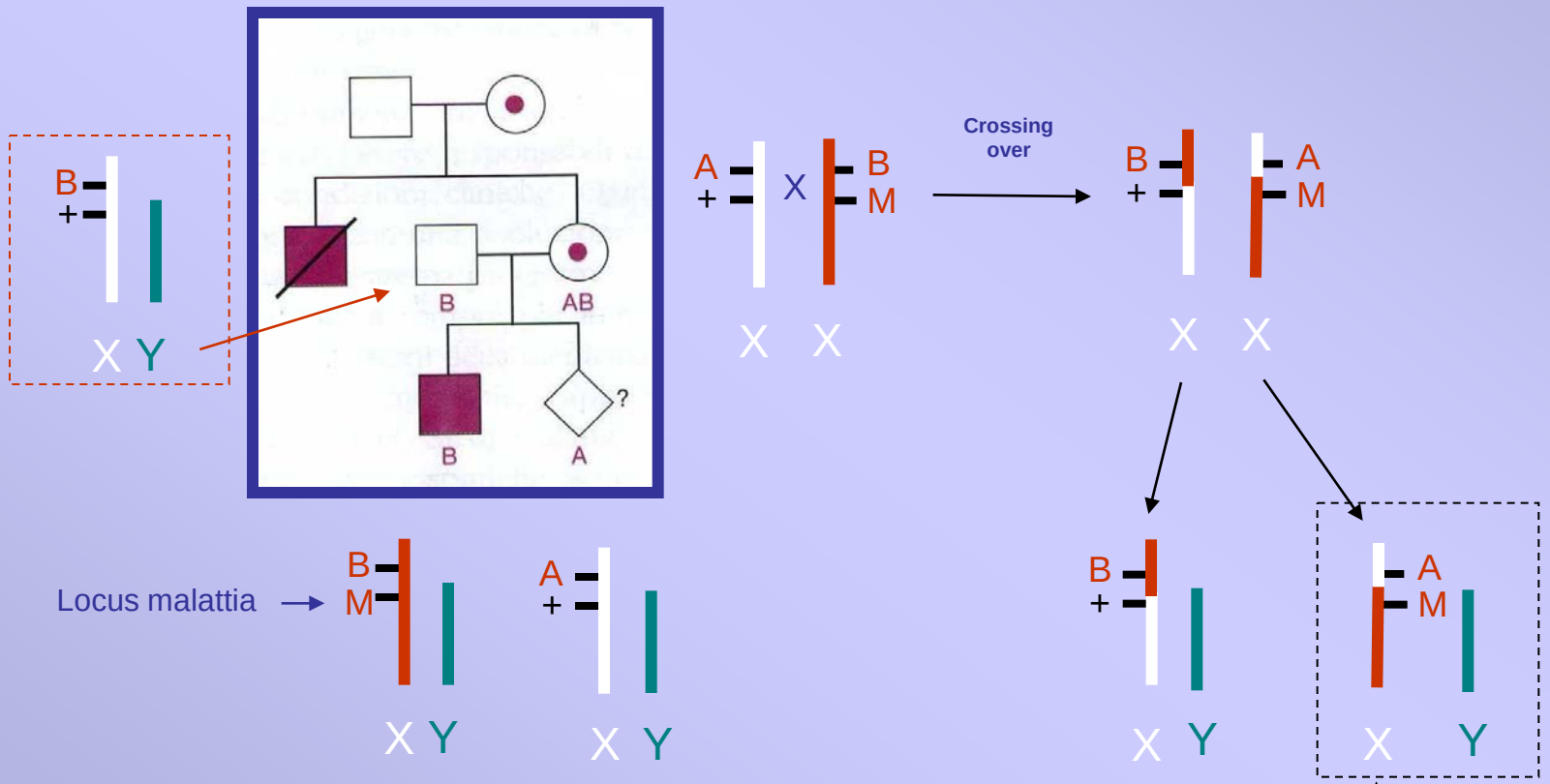
⇩

Calcolo del rischio

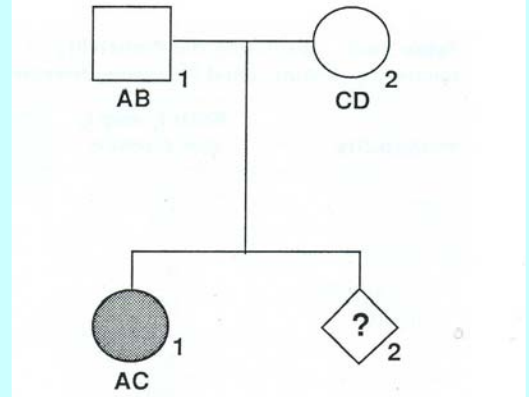
⇩

Analisi indiretta utilizzando marcatori del DNA vicini al gene

Analisi indiretta utilizzando marcatori del DNA vicini al gene



Analisi indiretta utilizzando marcatori del DNA vicini al gene



Ricombinazione possibile in entrambi i genitori……

AC → Affetto

AD

BC

BD

Analisi molecolare

- ✓ **Facilità di prelevare campioni da analizzare, approccio meno invasivo e rischioso**

- ✓ **Certezza della diagnosi di disordini in cui vi è un'alterazione di una proteina Tessuto specifica ed il tessuto è difficilmente ottenibile**

- ✓ **Individuazione con certezza degli eterozigoti per malattie autosomiche recessive**

- ✓ **Specificità dei risultati e possibilità di ottenere informazioni anche sulla gravità del fenotipo (Correlazione Genotipo-Fenotipo se definita)**
- ✓ **Migliore Pianificazione di una terapia**

- ✓ **Insostituibile per la Diagnosi prenatale (CVS, LA) specialmente per quelle patologie per le quali il gene, ed il difetto metabolico, non è espresso nei villi o negli amniociti**

- Terapia in utero o immediatamente alla nascita
- Scelta di interrompere la gravidanza

Analisi Molecolare

PCR

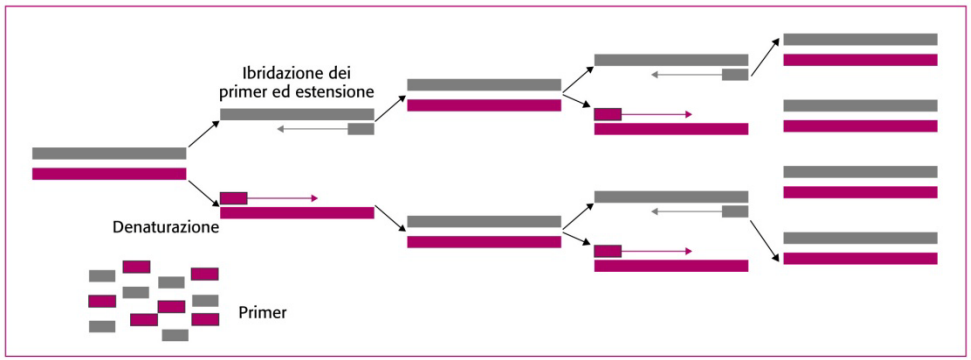


Figura 3.4 Rappresentazione schematica dell'amplificazione esponenziale di un frammento di DNA mediante reazione di PCR.

Neri G, Genuardi M. Genetica umana e medica. Elsevier Masson, Milano, 2007

Applicazioni della PCR

Ricerca di mutazioni note	Ricerca di mutazioni ignote
Delezioni • Omozigosi • Emizigosi	SSCP DGGE dHPLC
Alterazione di siti di restrizione	PTT
ASO	Sequenziamento diretto
ARMS	
OLA	
Controllo delle dimensioni di triplette ripetute	

ASO = Allele Specific Oligoprobe; ARMS = Amplification Refractory Mutation Systems; OLA = Oligonucleotide Ligation Assay; SSCP = Single Strand Conformation Polymorphism; DGGE = Denaturing Gradient Gel Electrophoresis; dHPLC = Denaturing High Performance Liquid Chromatography; PTT = Protein Truncation Test.

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Ricerca di mutazioni note

Delezioni
- Omozigosi
- Emizigosi

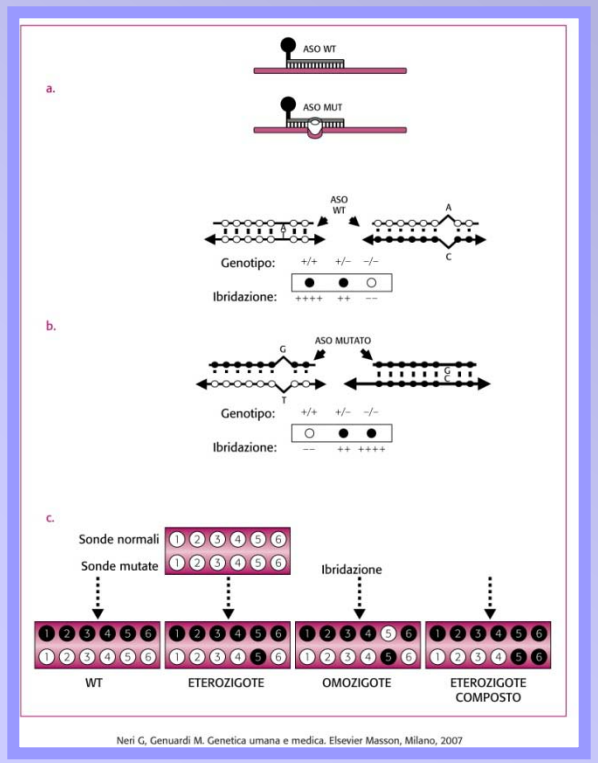
Alterazione di siti di restrizione

ASO

ARMS

OLA

Controllo delle dimensioni di triplette ripetute



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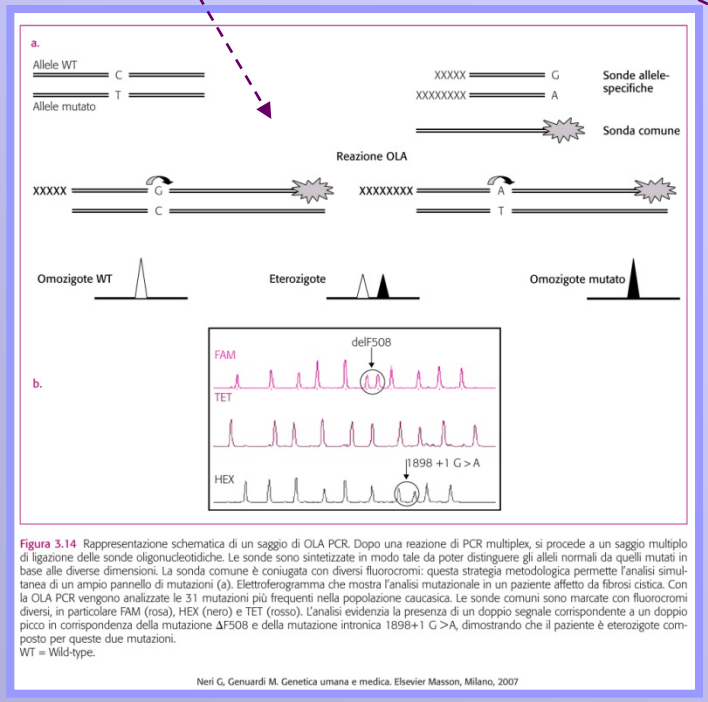
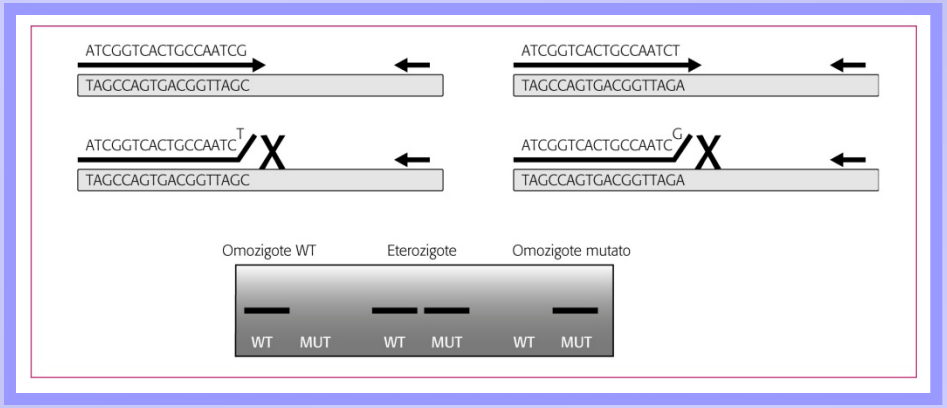


Figura 3.14 Rappresentazione schematica di un saggio di OLA PCR. Dopo una reazione di PCR multiplex, si procede a un saggio multiplo di ligazione delle sonde oligonucleotidiche. Le sonde sono sintetizzate in modo tale da poter distinguere gli alleli normali da quelli mutati in base alle diverse dimensioni. La sonda comune è coniugata con diversi fluorocromi: questa strategia metodologica permette l'analisi simultanea di un ampio pannello di mutazioni (a). Elettroferogramma che mostra l'analisi mutazionale in un paziente affetto da fibrosi cistica. Con la OLA PCR vengono analizzate le 31 mutazioni più frequenti nella popolazione caucasica. Le sonde comuni sono marcate con fluorocromi diversi, in particolare FAM (rosa), HEX (nero) e TET (rosso). L'analisi evidenzia la presenza di un doppio segnale corrispondente a un doppio picco in corrispondenza della mutazione ΔF508 e della mutazione intronica 1898+1 G >A, dimostrando che il paziente è eterozigote composto per queste due mutazioni.
WT = Wild-type.

Neri G, Genuardi M. Genetica umana e medica. Elsevier Masson, Milano, 2007



Ricerca di mutazioni ignote

SSCP
DGGE
dHPLC
PTT
Sequenziamento diretto

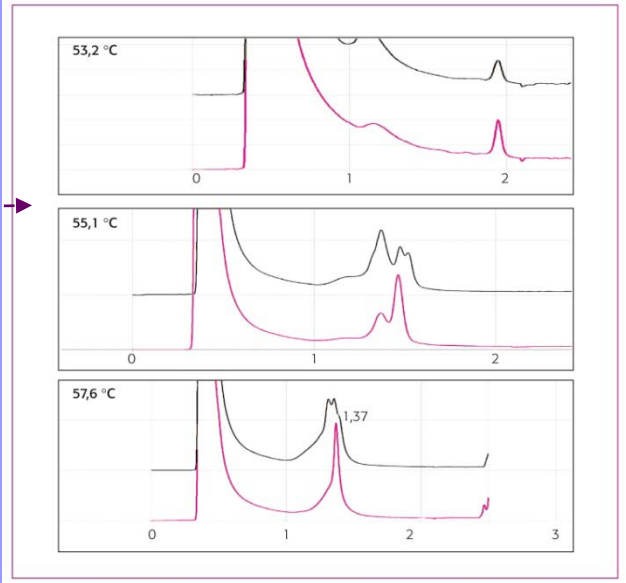


Figura 3.18 Screening mutazionale di un frammento dell'esone 11 del gene *BRCA2*. I cromatogrammi sono stati ottenuti con analisi dHPLC. Per ogni temperatura sono illustrate le curve di eluizione di un campione normale (in basso) e di un campione eterozigote per una mutazione (in alto). La mutazione è evidenziabile a due delle tre temperature di corsa (55,1 °C e 57,6 °C) come la presenza di un picco aggiuntivo.

Neri G, Genuardi M. Genetica umana e medica. Elsevier Masson, Milano, 2007

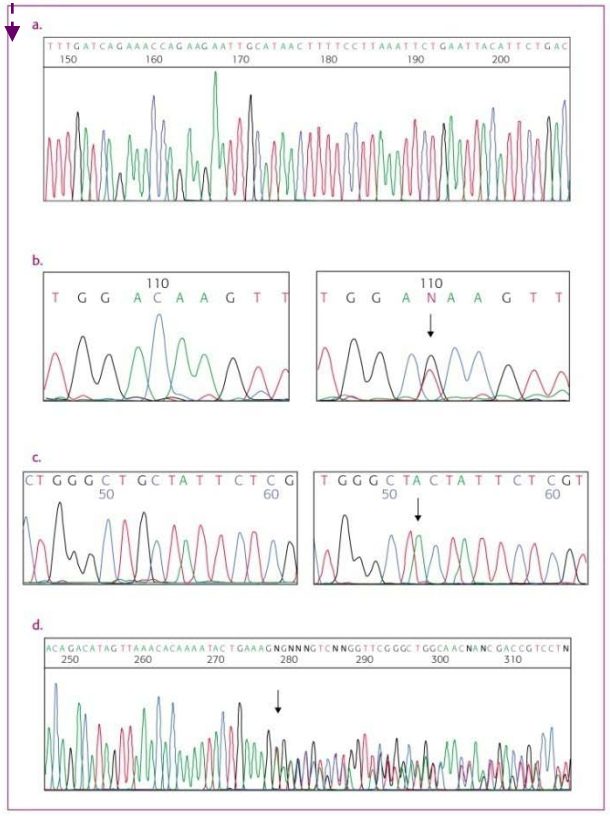


Figura 3.20 Esempio di un elettroferogramma ottenuto per sequenziamento automatico. Ogni picco, con uno pseudocolore diverso (rosso-timina, verde-adenina, nero-guanina, blu-citosina) corrisponde a una banda e quindi a una base direttamente rilevata dal software (a). Presenza di una mutazione puntiforme in eterozigosi (C > T): si evidenzia per la contemporanea presenza di due picchi nella stessa posizione (b). Presenza di una mutazione puntiforme in omozigosi (G > T) evidenziabile come la presenza di un picco singolo corrispondente al nucleotide mutato (c). Presenza di una mutazione frame-shift in eterozigosi. Dal punto mutato, indicato dalla freccia, si sovrappongono due diverse sequenze corrispondenti all'allele normale e a quello mutato (d).

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Deficit di OTC

Buona correlazione genotipo-fenotipo
> 200

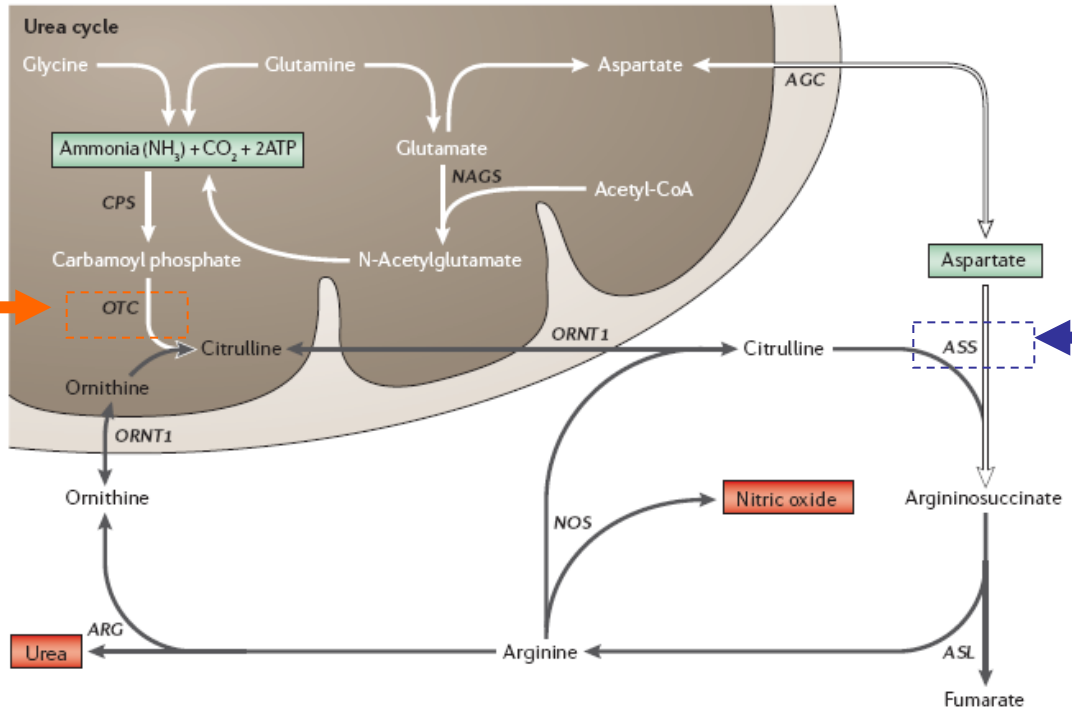
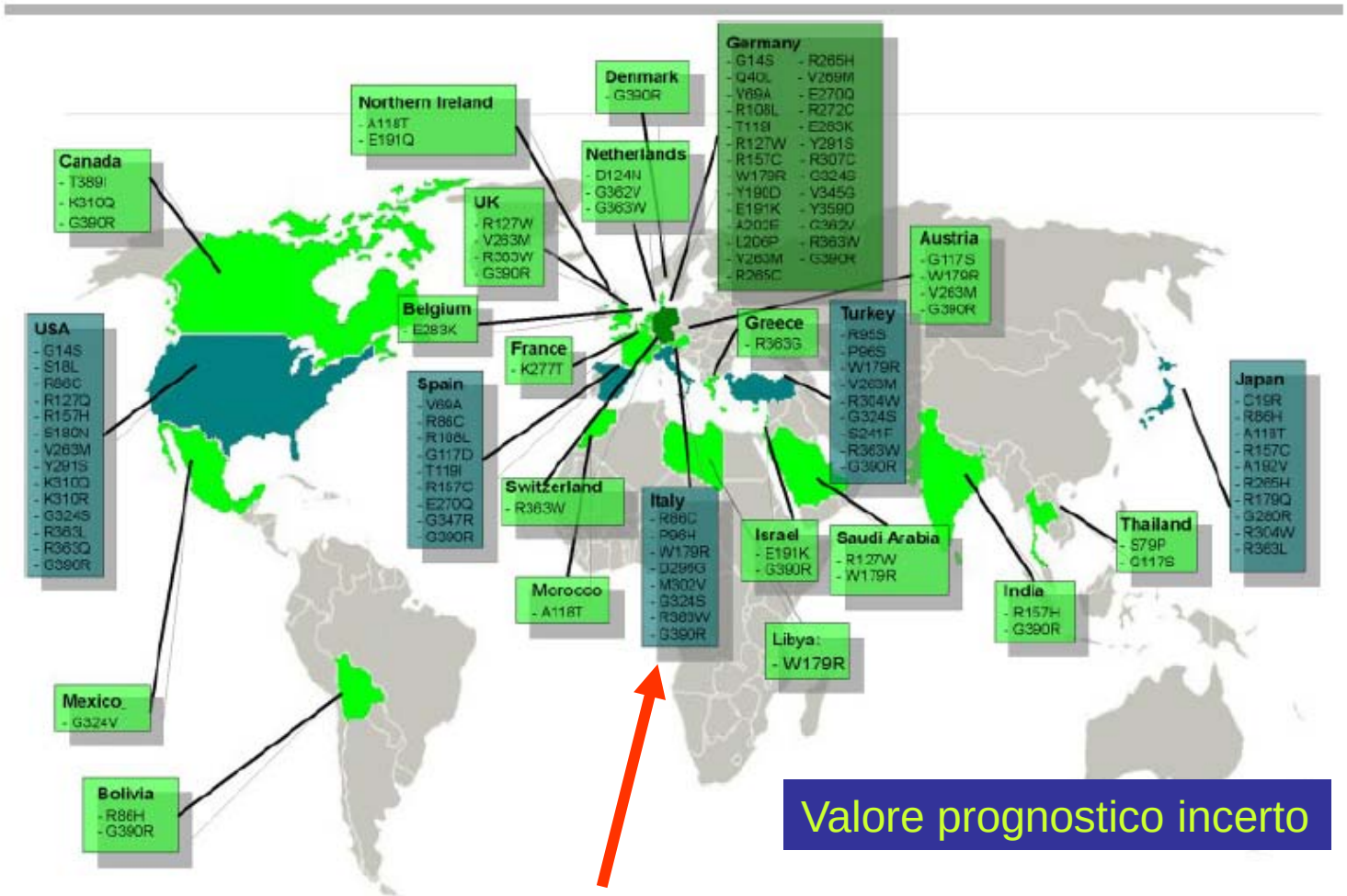


Figure 4 | **Urea-cycle disorders, small-molecule diseases.** The urea cycle consists of a group of enzymes that generate urea from nitrogen that is donated from ammonia and aspartate. Embedded within the urea cycle is the arginine–citrulline cycle, which produces nitric oxide. Disease-causing mutations have been identified in all enzymes and transporters shown here. The most common urea-cycle disorder is ornithine transcarbamylase deficiency (OTCD, OMIM 311250). The other urea-cycle disorders are deficiencies of N-acetyl glutamate synthetase (NAGS, OMIM 237310), carbamyl phosphate synthetase (CPS, OMIM 237300), argininosuccinic acid synthetase (ASS, OMIM 215700), argininosuccinic acid lyase (ASL, OMIM 207900, also known as argininosuccinic aciduria (ASA)), and arginase I (ARG1, OMIM 207800). AGC, aspartate-glutamate carrier (citrin); NOS, nitric-oxide synthase; ORNT1, ornithine translocase-1.

Citrullinemia di tipo I

Valore prognostico ancora incerto delle Mutazioni genetiche
Finora identificate 87

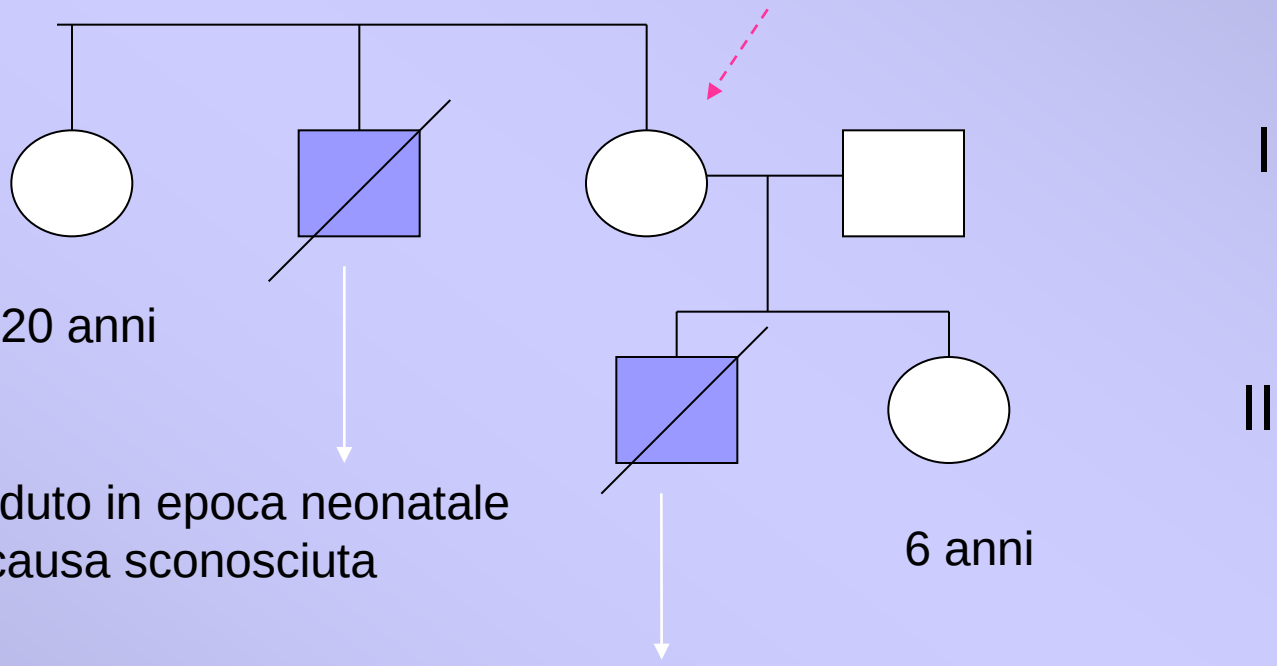
Deficit di Argininosuccinato sintetasi

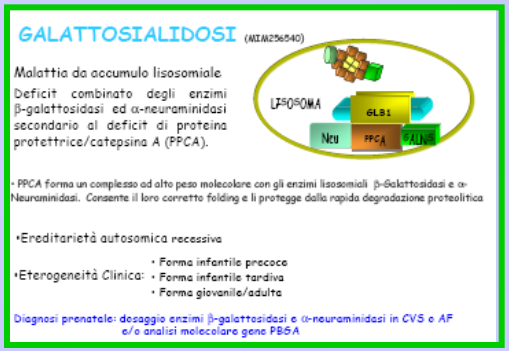


Valore prognostico incerto

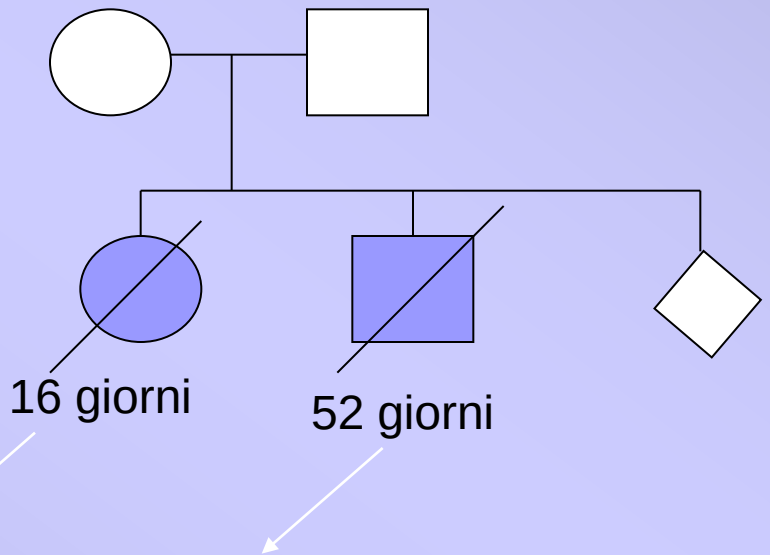
Figure 3. Geographical distribution of ASS missense mutations. Color code: light green, countries in which one to seven different mutations have been described; turquoise, countries with eight to 19 mutations; and dark green, countries with 20 or more mutations. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Deficit di OTC





L S D



Idrope generalizzata

Galattosialidosi precoce infantile

HFI
(Intolleranza ereditaria al fruttosio)

Procedure invasive per la diagnosi prima del clonaggio del gene

Analisi mutazionale fattibile e avvantaggiata dalla conoscenza dell'epidemiologia molecolare

La ricerca delle mutazioni più frequenti è in grado di confermare la diagnosi quasi nel 100% dei casi

Tabella 4-2. Mutazioni del gene dell'aldolasi B in 19 pazienti italiani con intolleranza ereditaria al fruttosio
(da Santamaria e coll., J Med Genet 33: 786-788, 1996)

Mutazione	Frequenza allelica %
A174D	29
A149P	29
MDΔ4	18,5
N334K	5,2
Y203X	5,2
R303W	5,2
ΔA20	5,2
L256P	2,7

Genotipo	Frequenza del genotipo %
A149P/A174D	21
A149P/A149P	10,5
A174D/ΔA20	10,5
A174D/MDΔ4	10,5
A149D/MDΔ4	10,5
A174D/A174D	5,3
MDΔ4/MDΔ4	5,3
MDΔ4/N334K	5,3
N334K/L256P	5,3
R303W/R303W	5,3
A174D/Y203X	5,3
A149P/Y203X	5,3

Buona Correlazione
Genotipo-Fenotipo

GD
Malattia di Gaucher

➢200 Mutazioni in GBA(deficit di glucocerebrosidasi)
➢ in rari casi del suo attivatore saposina C

tipo 1 è la forma cronica e non neurologica 95 % dei casi →

tipo 2 è la forma neurologica acuta 1 % →

tipo 3 è la forma neurologica subacuta 4% →

Due Mutazioni

N307S e L444P
hanno valore predittivo

Genetic medicines: treatment strategies for hereditary disorders

Timothy P. O'Connor and Ronald G. Crystal

Abstract | The treatment of the more than 1,800 known monogenic hereditary disorders will depend on the development of 'genetic medicines' — therapies that use the transfer of DNA and/or RNA to modify gene expression to correct or compensate for an abnormal phenotype. Strategies include the use of somatic stem cells, gene transfer, RNA modification and, in the future, embryonic stem cells. Despite the efficacy of these technologies in treating experimental models of hereditary disorders, applying them successfully in the clinic is a great challenge, which will only be overcome by expending considerable intellectual and economic resources, and by solving societal concerns about modifications of the human genetic repertoire.

Metabolic manipulation
The use of dietary modification or small molecule therapy to compensate for a deranged biological process.

Protein augmentation
A therapy in which a missing protein is replaced by the administration of a protein that has been purified from mammalian cells/tissues or synthesized as recombinant protein.

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doi:10.1038/nrg1829

With the sequencing of the human genome and the concomitant understanding of genotype–phenotype relationships, increasing attention has been paid to applying this knowledge to treating inherited diseases. Whereas strategies such as metabolic manipulation and protein augmentation have been remarkably successful in treating some genetic diseases (BOX 1), the real therapeutic breakthroughs for hereditary disorders will depend on the development of 'genetic medicines': therapies that are centred on transferring genetic material to correct or compensate for an abnormal phenotype associated with a particular genotype.

Of the approximately 25,000 genes that comprise the human genome, mutations in more than 1,800 have already been identified as causing hereditary disorders (Ensembl, OMIM). The focus of this review is the use of genetic medicines to treat monogenic hereditary disorders. Because so many single gene mutations are known, the logic is compelling that if sufficient correction or compensation can be achieved with genetic medicines, monogenic disorders could be prevented and/or treated. By contrast, current genetic medicines cannot correct the complex phenotype associated with the hundreds of genes that are typically affected in chromosomal disorders (such as trisomy 21) or the multiple genetic variations that underlie complex disorders. For these disorders, strategies are being developed to compensate for, or to modify, diseased organs. Examples include gene therapy to induce angiogenesis to bypass blocked coronary arteries, or stem cell therapy to regenerate cardiac myocytes to treat a failing myocardium.

Three broad categories of genetic medicines are being tested in the clinic: somatic stem cells (SSCs), gene transfer and RNA modification. In the future, the application of embryonic stem cells (ESCs) will be assessed. For each strategy, the fundamental approach is to modify the gene expression repertoire of a subset of somatic cells/organs of the affected individual. No current strategy targets the germ line. The different categories of genetic medicine are not mutually exclusive, and it is likely that genetic medicines of the future will include various combinations of these approaches.

Genetic medicines are simple in concept, but challenging to make a therapeutic reality. We first outline the general concepts that are applicable to genetic medicines. We then review the genetic medicine strategies being developed to treat monogenic disorders, including those that involve the use of SSCs (excluding combined SSC–gene-transfer strategies, which are discussed in the section on gene transfer), gene transfer, RNA modification, and ESCs. For each of these strategies we describe the current status of applying these therapies to treat hereditary human disorders and the biological challenges in making genetic medicine therapies a reality. Finally, we discuss the future of genetic medicines, including the regulatory, economic and social hurdles in developing genetic medicines. To provide a historical context, strategies for treating these disorders in the pre-genetic medicine era are summarized in the Supplementary information S1–S3 (tables).

Genetic medicines — general considerations

The concept underlying all genetic medicines is that transfer of genetic material (for example, coding for a

“ Pre-genetic medicine ”

Box 1 | **Treating hereditary disorders in the pre-genetic medicine era**

Before the development of genetic medicines, strategies for treating hereditary disorders focused on metabolic manipulation and protein augmentation therapy. For some monogenic disorders, success with these approaches has been remarkable.

Metabolic manipulation

The basic concept of metabolic manipulation is to use dietary or small molecule therapy to compensate for a deranged biological process, or in some instances, to prevent the complications of therapies used to correct the abnormal phenotype (see Supplementary information S2 (table)) for a summary and references). The simplest form of metabolic manipulation is diet modification (such as phenylalanine restriction to treat phenylketonuria). For some disorders, successful therapy depends on combining diet manipulation with drugs (for example, for familial hypercholesterolaemia this involves the combination of a low-cholesterol diet and statin inhibitors of hydroxymethylglutaryl co-enzyme A (HMG CoA) reductase. Another strategy involves stimulating the expression of a protein that will substitute for the abnormal protein — for example, hydroxyurea drug therapy is used to stimulate expression of fetal haemoglobin (HbF, $\alpha_2\gamma_2$) levels to compensate for the abnormal sickle cell haemoglobin (HbS, $\alpha_2\beta_2$), therefore reducing the sickle crises of sickle cell anaemia. Alternatively, if the mutant protein is functional it can be effective to upregulate the expression of the mutant protein; an example is 'impeded' androgen therapy for C1-inhibitor deficiency, which is an autosomal dominant disorder that causes hereditary angioedema. Metabolic therapies can also be used to treat the complications from the treatment of genetic disorders, such as treatment of thalassaemia with the iron-chelating agent desferrioxamine; this drug prevents the organ failure that would otherwise be caused by the iron overload from the frequent red blood cell transfusions that are required to treat the primary phenotype. High-throughput screening of chemical libraries is being used to identify small molecules that modify the conformation of misfolded mutant proteins, which enables mutant proteins to traffic and/or function normally, or otherwise trick chaperones and other organelle-specific quality-control systems to accept the misfolded protein.

Protein augmentation

The concept of protein augmentation therapy is simple — purify the missing protein and return it to the patient. This therapy is used in several hereditary disorders, including cystic fibrosis, coagulation disorders, α1-antitrypsin deficiency, immunoglobulin deficiencies, endocrine disorders and lysosomal storage diseases (see Supplementary information S3 (table)). Protein augmentation therapy is most applicable to treating hereditary disorders in which the deficient protein functions in the extracellular milieu. If the protein has to reach sites from which it was prevented from diffusing (such as the brain), systemic protein augmentation therapy is not effective[xxxx]. When the phenotype involves an intracellular protein, protein augmentation therapy can be effective only if there is a mechanism to import the protein into a compartment of the cell relevant to the abnormal phenotype, such as protein augmentation therapy for the lysosomal storage disorders[xxxx]. Other challenges for treating hereditary disorders with protein augmentation therapy include: maintaining venous access to administer the protein; infection; supply shortages of the therapeutic agent; cost; requirement of frequent, repeated administrations; and the potential allergic, inflammatory and immune responses to the infused proteins[xxxx].

Type of therapy	Disorder	Abnormal gene	Treatment	Outcome	References[2]
Dietary ± drugs to modify metabolic pathways	Phenylketonuria	Phenylalanine hydroxylase	Lifelong restriction of dietary ph… su…	Normal development, …	1,2
	Galactosemia	Galactose-1-phosphate uridyltransferase	Li… an…		
	Lactose intolerance	Lactase-phlorizin hydrolase	A… ad…		
	Hereditary fructose intolerance	Aldolase B	El… an…		
	Maple syrup urine disease	Branched-chain α-keto acid dehydrogenase	Li… di… ac…		
	Familial hypercholesterolemia	Low density lipoprotein receptor	A… ch… m…		
	Glycogen storage disease I	Glucose-6-phosphatase	Fr… gl…		
	Hereditary orotic aciduria	UMP synthase[3]	La…		
	Phosphoglycerate dehydrogenase deficiency	Phosphoglycerate dehydrogenase	O… supplements		3

Figura 1.11 Le donne in questa fotografia sono sorelle. Entrambe sono omozigoti per la stessa mutazione nel gene per la fenilalanina idrossilasi (*PAH*). La sposa (la più giovane delle due), a cui la PKU è stata diagnosticata tre giorni dopo la nascita, è stata sottoposta subito alla dieta. Alla damigella d'onore, la sorella più vecchia, la patologia è stata diagnosticata troppo tardi e la dieta non ha portato benefici.

		factor		corrected	
Emphysema	α1-antitrypsin deficiency	α1-antitrypsin	Pooled human plasma	Decreased mortality from emphysema	8,9
Immune deficiency	Severe combined immune deficiency	Adenosine deaminase	Bovine adenosine deaminase coated with polyethylene glycol	Reduced incidence of infections	10
Endocrine disorders	Growth hormone deficiency	Growth hormone	Recombinant growth hormone	Normal growth	11
	Congenital leptin deficiency	Leptin	Recombinant leptin	Normalization of hyperphagia; reduction in body mass	12
	Congenital neurogenic diabetes insipidus	Anti-diuretic hormone	Recombinant anti-diuretic hormone	Abolishment of polyuria and polydipsia	13

S3 | Reports of correction of hereditary disorders by transplantation of unmodified non-autologous bone marrow[1]

Category	Disorder	Affected gene	Outcome	References[2]
Lysosomal storage disorders	Mucopolysaccharidosis type I (Hurler syndrome)	α-L-iduronidase	Significant substrate reduction in CNS and peripheral organs; bone deformities and corneal clouding persist	1,2
	Mucopolysaccharidosis type II (Hunter syndrome)	Idurondate-2-sulfatase	Marginal improvement in visceral pathology; no effect on CNS degeneration	3
	Mucopolysaccharidosis type III (Sanfilippo syndromes)	Different genes affected in different forms[3]	No evidence of long-term slowing of cognitive decline	4,5
	Mucopolysaccharidosis type IV (Morquio syndromes)	Different genes affected in different forms[4]	Failed to improve skeletal abnormalities	5
	Mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome)	Arylsulfatase B	Improved cardiopulmonary function and organomegaly, but not neurologic function; bone deformities persist	6
	Mucopolysaccharidosis type VII (Sly disease)	β-glucuronidase	Increased β-glucuronidase activity in lympocytes; improvements in motor function	7
	Gaucher disease	Glucocerebrosidase	Favorable psychological development, clearance of Gaucher cells in the liver, spleen and lungs	8
	Niemann-Pick A and B	Sphingomyelinase	Decreased accumulation of sphingomyelin in liver, spleen and bone marrow; no effect on CNS degeneration in type A	9,10
	Aspartylglucosaminuria	Aspartylgluco-aminidase	Increased aspartylglucosaminidase activity in peripheral blood leukocytes; phenotype not improved over long-term	11,12

Gene transfer

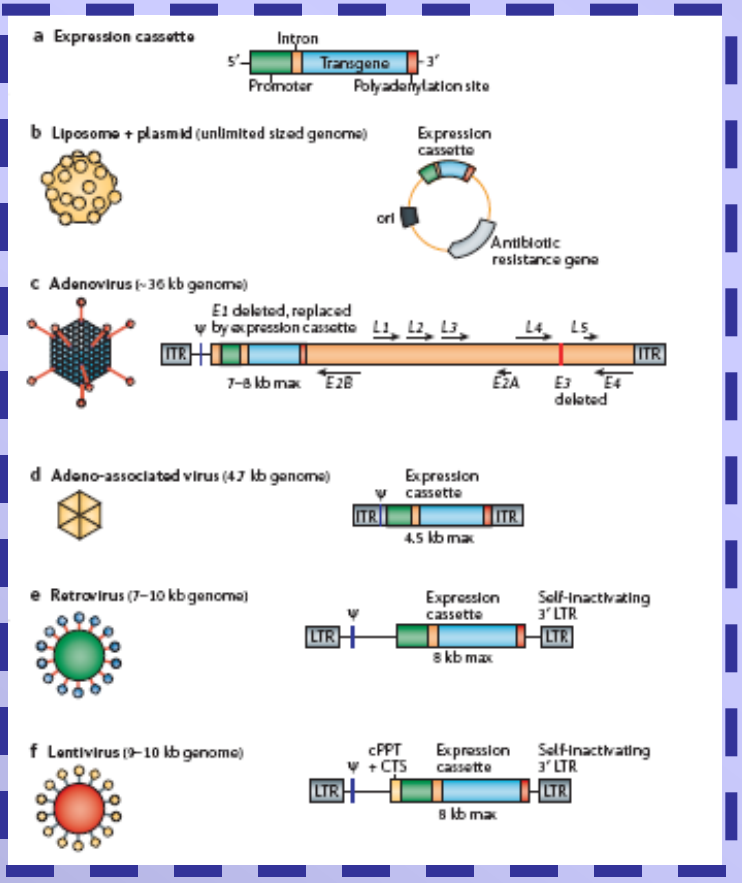
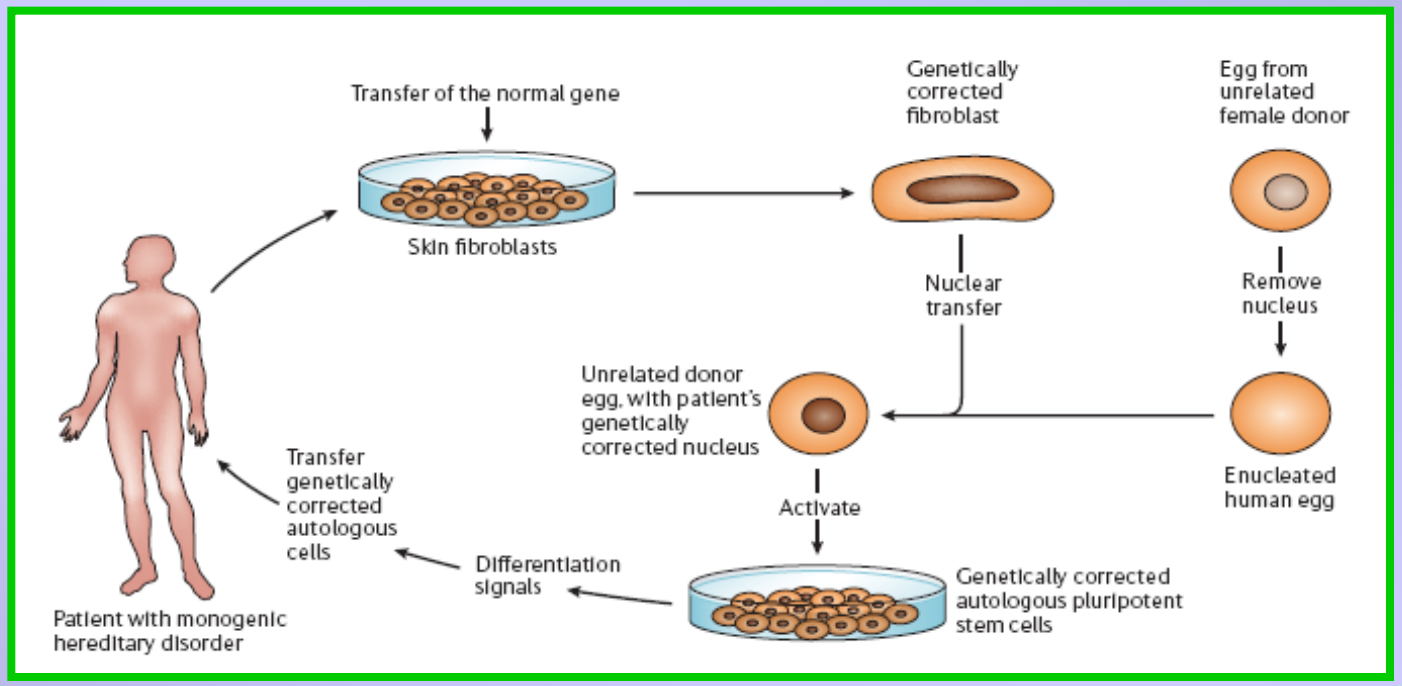


Table 1 | Gene-transfer trials for monogenic hereditary disorders*

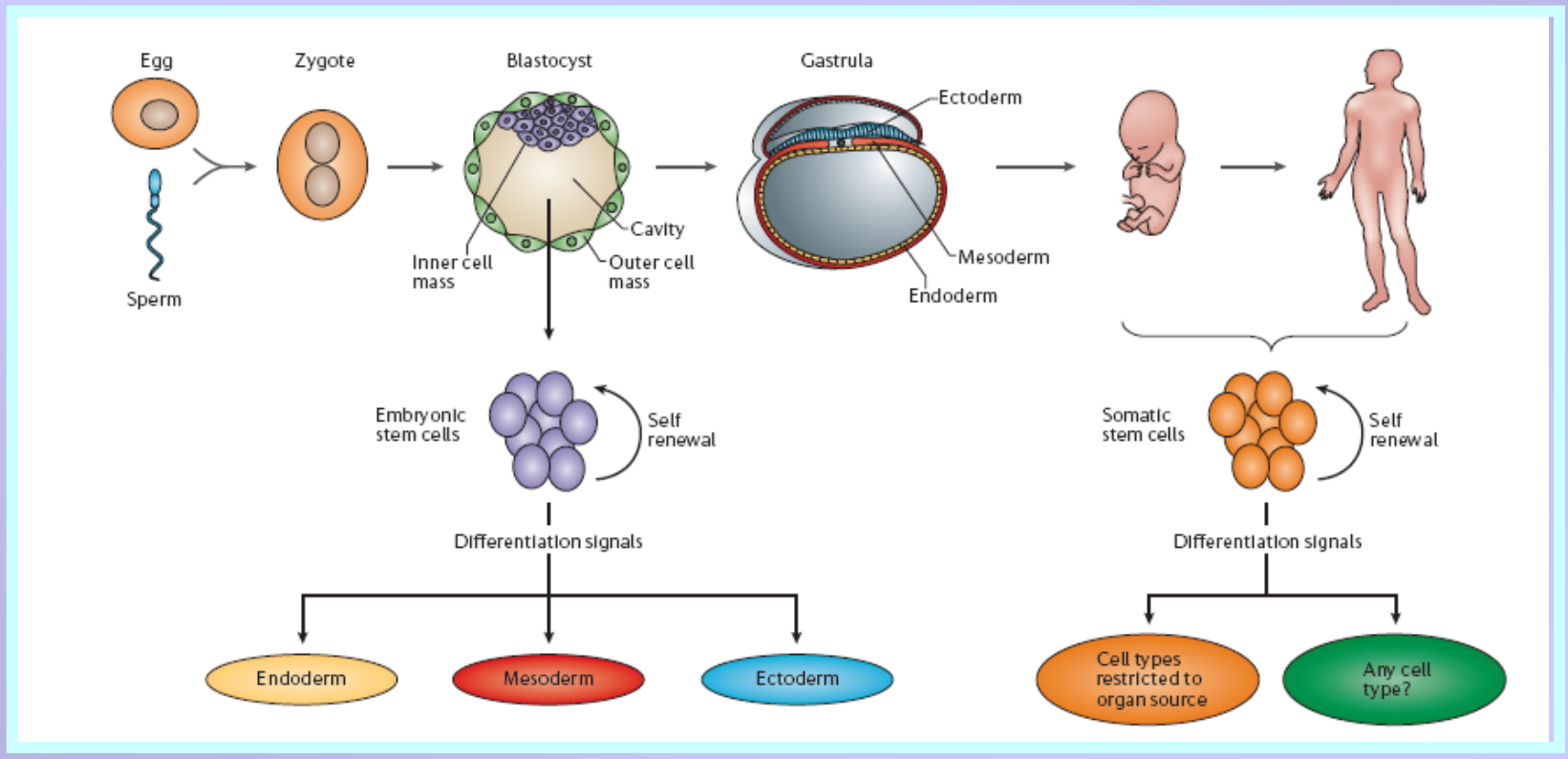
Vector	Hereditary disorder (references[§])	Transgene	Target cells	Evidence for phenotype correction[‡]		
				In vivo GBI (mRNA)	*In vivo* biochem., physiological or imaging methods	CI[¶]
Plasmid ±; liposome (*ex vivo*)	Haemophilia A (136)	Factor VIII	Fibroblasts	NA	±	±
Plasmid ±; liposome (*in vivo*)	Cystic fibrosis (137–144)	CFTR	Nasal and airway epithelium	±	±	No
	α1-antitrypsin deficiency (145)	α1-antitrypsin	Nasal and respiratory tract epithelium	±	No	No
	Canavan disease (146)	Aspartoacylase	CNS	?	?	?
	Muscular dystrophy (147)	Dystrophin	Muscle	±	No	No
Retrovirus (*ex vivo*)	Adenosine deaminase deficiency (17,75,77,78,148,149)	Adenosine deaminase	T cells, CD34 cells, cord blood, bone marrow	YES	YES	YES
	Familial hypercholesterolaemia (74,150)	Low-density lipoprotein receptor	Hepatocytes	Yes	Yes	No
	Gaucher disease (151)	Glucocerebrosidase	Blood CD34+, bone marrow CD34+	±	No	No
	Fanconi anaemia (152,153)	Complementation group C or A	Blood CD34 cells	±	No	No
	Chronic granulomatous disease (154)	p47 phagocyte NADPH oxidase	Blood CD34+	±	No	No
	X-linked severe combined immunodeficiency (80–82,155)	Common γ-chain of multiple cytokine receptor	Cord blood and bone marrow CD34+	YES	YES	YES
	Leukocyte adherence deficiency (156,157)	CD18	Blood CD34+	±	No	No
	Severe combined immunodeficiency secondary to JAK3 deficiency (158)	JAK3	Bone marrow CD34+	?	?	?
	Haemophilia B (159)	Factor IX	Skin fibroblasts	NA	±	No
Retrovirus (*in vivo*)	Haemophilia A (160)	Factor VIII	Intravenous[#]	±	±	No
Adenovirus serotypes 2 and 5** (*in vivo*)	Cystic fibrosis (32,37, 42–45,161,162)	CFTR	Nasal and airway epithelium	Yes	Yes	No
	Ornithine transcarbamylase deficiency (49,163)	Ornithine transcarbamylase	Liver	±	±	No
	Haemophilia A (50)	Factor VIII	Liver	±	±	No
Adeno-associated virus serotype 2 (*in vivo*)	Cystic fibrosis (67,164–166)	CFTR	Nasal, airway and maxillary sinus epithelium	No	No	No
	Haemophilia B (66,167; see the ASGT Stakeholder's Report in the Further information)	Factor IX	Muscle, liver	Yes	Yes	No
	Muscular dystrophy (66)	α,β,γ,Δ-sarcoglycan	Muscle	?	?	?
	Canavan disease (146,168)	Aspartoacylase	CNS	?	?	?
	Late infantile ceroid lipofuscinosis (169)	*CLN2* (tripeptidyl peptidase 1)	CNS	?	?	?

Lista dei trials approvati su :
"Gene Therapy Clinical Trials Worldwide " web site (www.wiley.co.uk/genetherapy/clinical) e su " the Clinical Trials in Human Gene Transfer " web site (www4.od.nih.gov/oba/rac/clinicaltrial.htm)

Modello per "Genetic Medicine"
Gene transfer + Somatic cell nuclear transfer+ Stem cell technologies



Embryonic Stem Cells e Somatic Stem Cells



PKU…..eterogeneità allelica e di locus

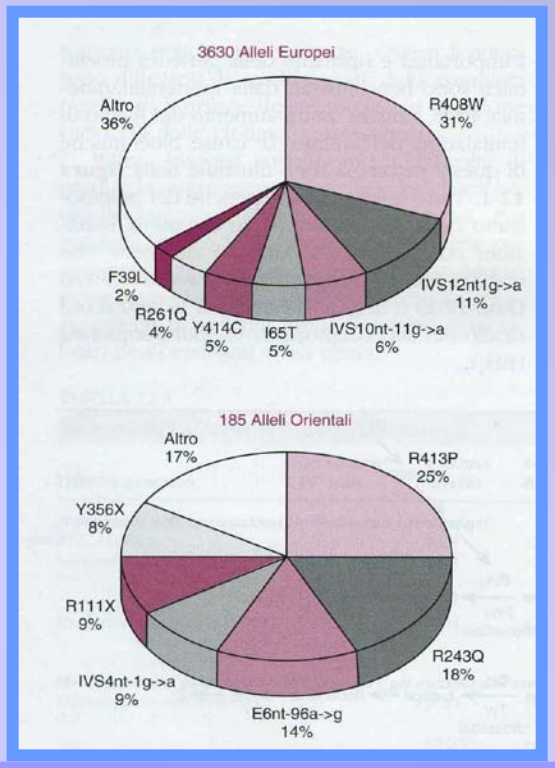
TABELLA 12-3

Eterogeneità al locus dell'iperfenilalaninemia

Difetto genetico	Incidenza/ 10^6 nati	Enzima affetto	Localizzazione del gene	Ereditarietà	Trattamento
Mutazioni nell'apoenzima fenilalanina idrossilasi					
PKU classica	5-350	PAH	12q24.1	AR	Dieta con poca fenilalanina
Varianti della PKU	meno della PKU classica	PAH	12q24.1	AR	Dieta con poca fenilalanina (meno restrittiva di quella richiesta per curare i pazienti PKU)
Iperfenilalaninemia non-PKU	15-75	PAH	12q24.1	AR	Nessuno o dieta con poca fenilalanina non restrittiva
Mutazioni nei geni che codificano per gli enzimi del metabolismo della tetraidrobiopterina					
Danneggiato riciclo di BH_4	1-2	PCD	10q22	AR	Dieta con poca fenilalanina più L-dopa, 5-HT, carbidopa
		DHPR	4p15.31		Dieta con poca fenilalanina più L-dopa, 5-HT, carbidopa e acido folico
Danneggiata sintesi di BH_4		GTP-CH	14q22	AR	Dieta con poca fenilalanina più L-dopa, 5-HT, carbidopa, acido folico e un dose farmacologica di BH_4
		6-PTS	11q22.3-23.3	AR	Come sopra

5-HT = 5-idrotriptofano; 6-PTS = 6-piruvoiltetraidropterina sintetasi; BH_4 = tetraidrobiopterina; DHPR = diidropteridina riduttasi; GTP-CH = guanosina trifosfato cicloidrolasi; PAH = fenilalanina idrolasi; PCD = pterin 4-α-carbinolammina riduttasi; PKU = fenilchetonuria.

PKU…..eterogeneità allelica e di locus



Il gene della fenilalanina idrossilasi: mutazioni, aplotipi e fenotipi clinici nella popolazione di discendenza europea

Mutazioni	% di attività	Rappresentazione delle mutazioni*	Aplotipo associato†	Fenotipo associato all'omozigote‡
Arg 408 Trp	<1%	31%	1, 2, 5, 44	Classica PKU
IVS12nt1g → a§	<1%	11%	3	Classica PKU
IVS10nt 2 11g → a	non nota	6%	6, 10, 34, 36	Classica PKU
Ile 65 Thr	~25%	5%	9	Classica PKU, Variante PKU, Non-PKU HPA
Tyr 414 Cys	30-50%¶	5%	4	Variante PKU, Non-PKU HPA
Arg 261 Gln	30%¶	4%	1, 2, 4, altri	PKU o variante PKU