Patologia genetica y molecular

'enfermedades del metabolismo', 'acumulo' 'depositos'
- proteinas – lipidos – carbohidratos – pigmentos - minerales

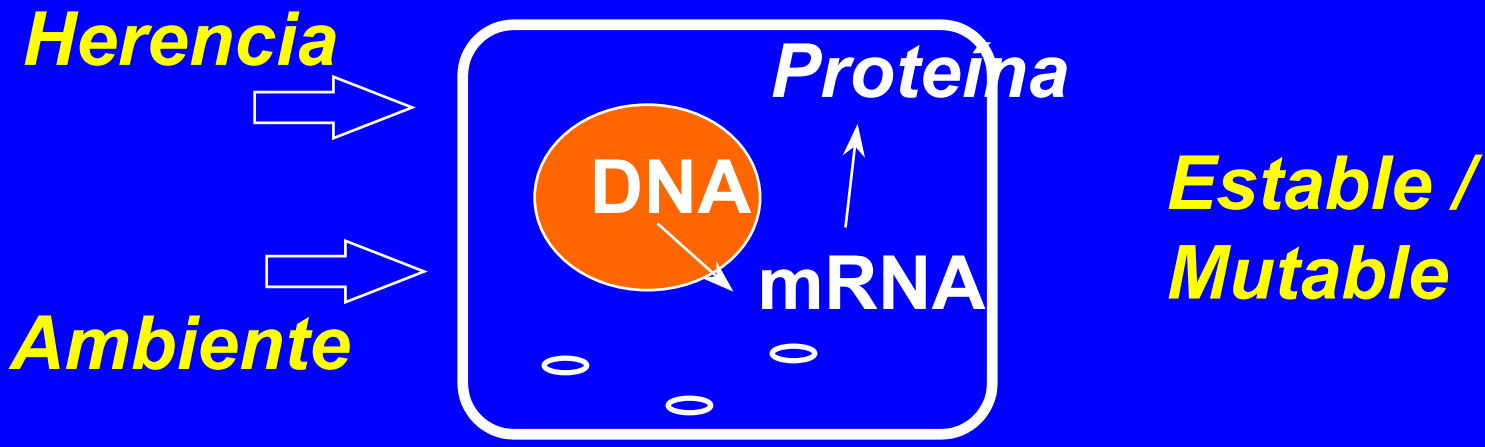
0. Anomalias cromosomicas (Citogenetica)
1. Enf. Mendelianas (monogenicas):
   - enzimas: trast.congenitos metabolismo
   - receptores y trasportadores
   - proteinas estructurales

2. Enf. Multifactoriales: diabetes, amiloidosis, gota

3. Neoplasias (genetica de linea somatica)

Ref.: Cotran, Kumar, Collins: Bases patologicas de la Enfermedad, 6 ed.
Cap. 2, 6, 7, 8, 11, 19, 20, 28, 30

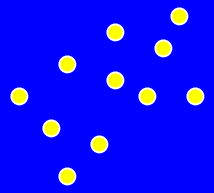
Flujo de información



- *células germinales*
- *células somáticas*

GENÉTICA MOLECULAR DE LAS NEOPLASIAS

crecimiento — diferenciacion — apoptosis



HIPERPLASIA

NEOPLASIA BENIGNA

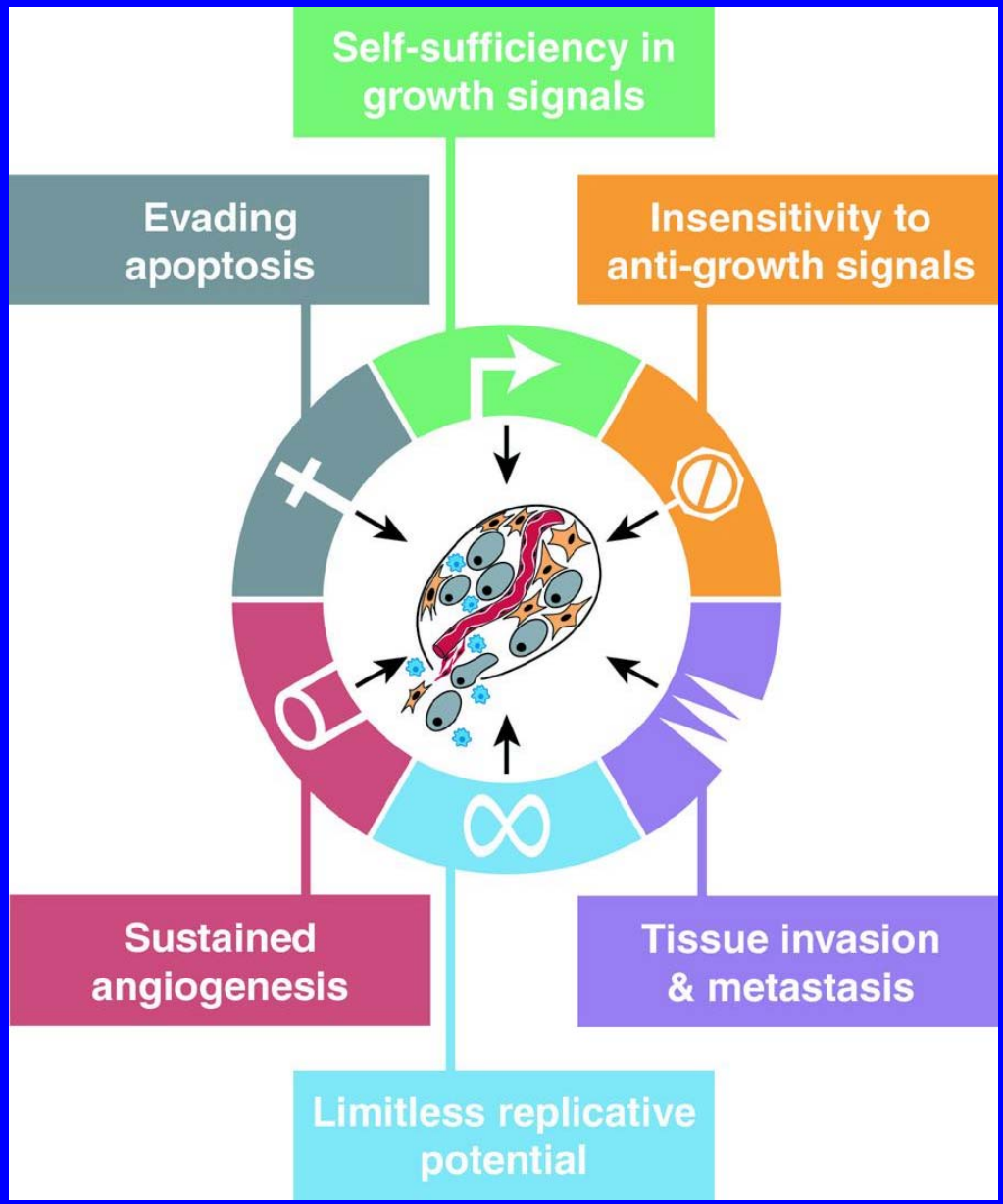
NEOPLASIA MALIGNA

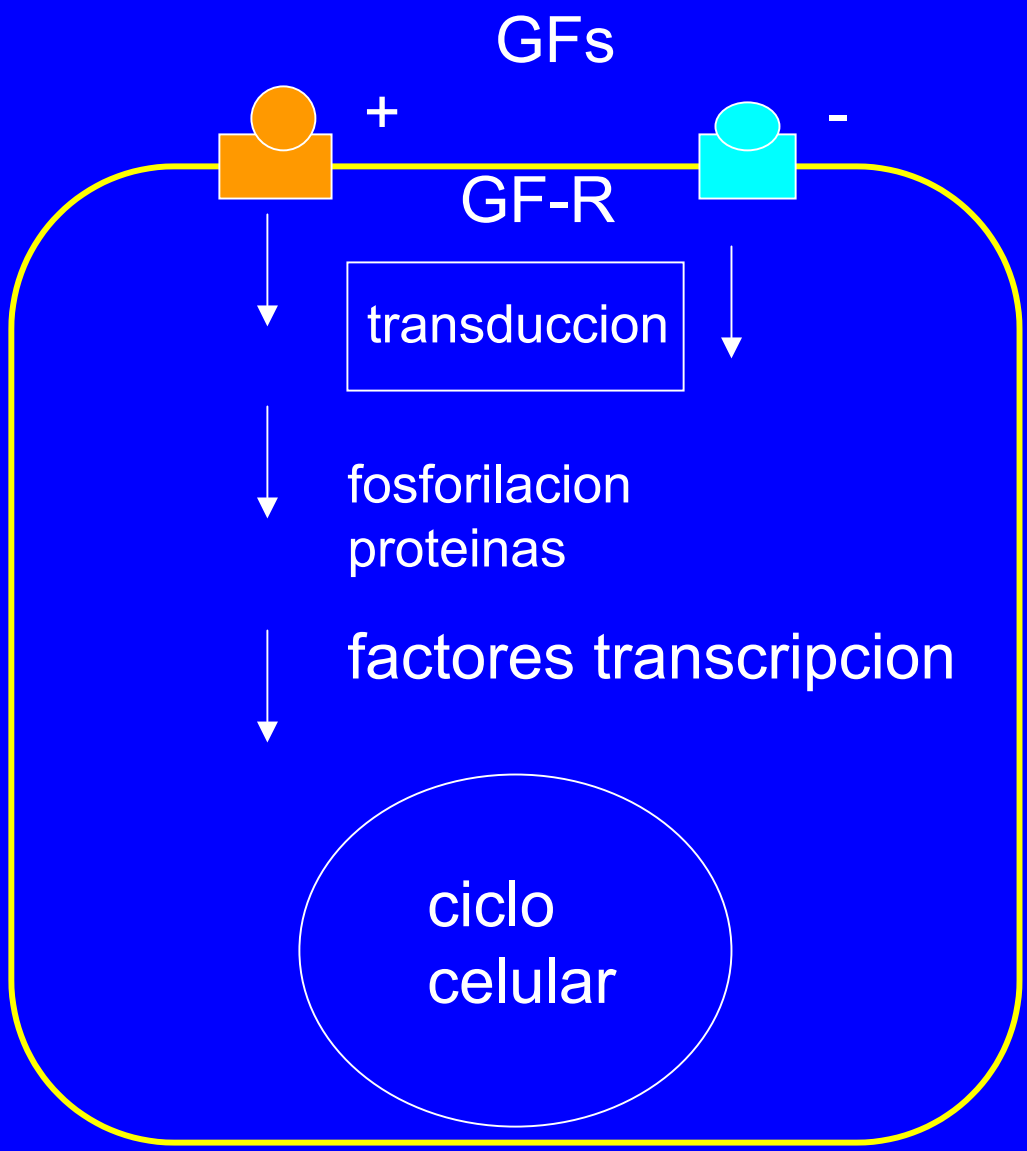
METASTASIS

Multiples pasos
Mutaciones somaticas
(germinales)
Clonal
Seleccion

<u>Factores etiologicos:</u>
-Hereditarios
-Hormonas
-Mutagenos
-Virus

Requerimientos de un cáncer

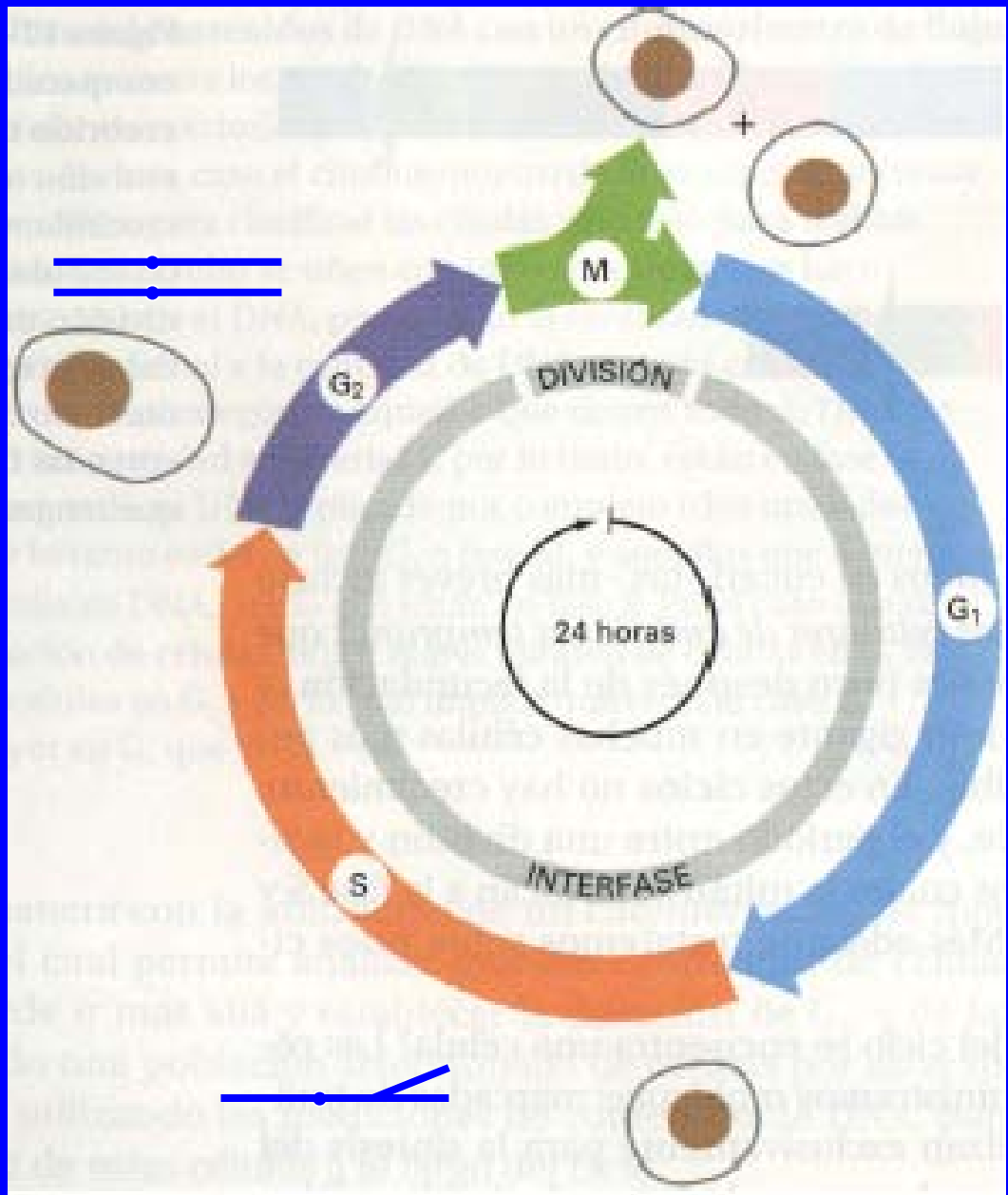




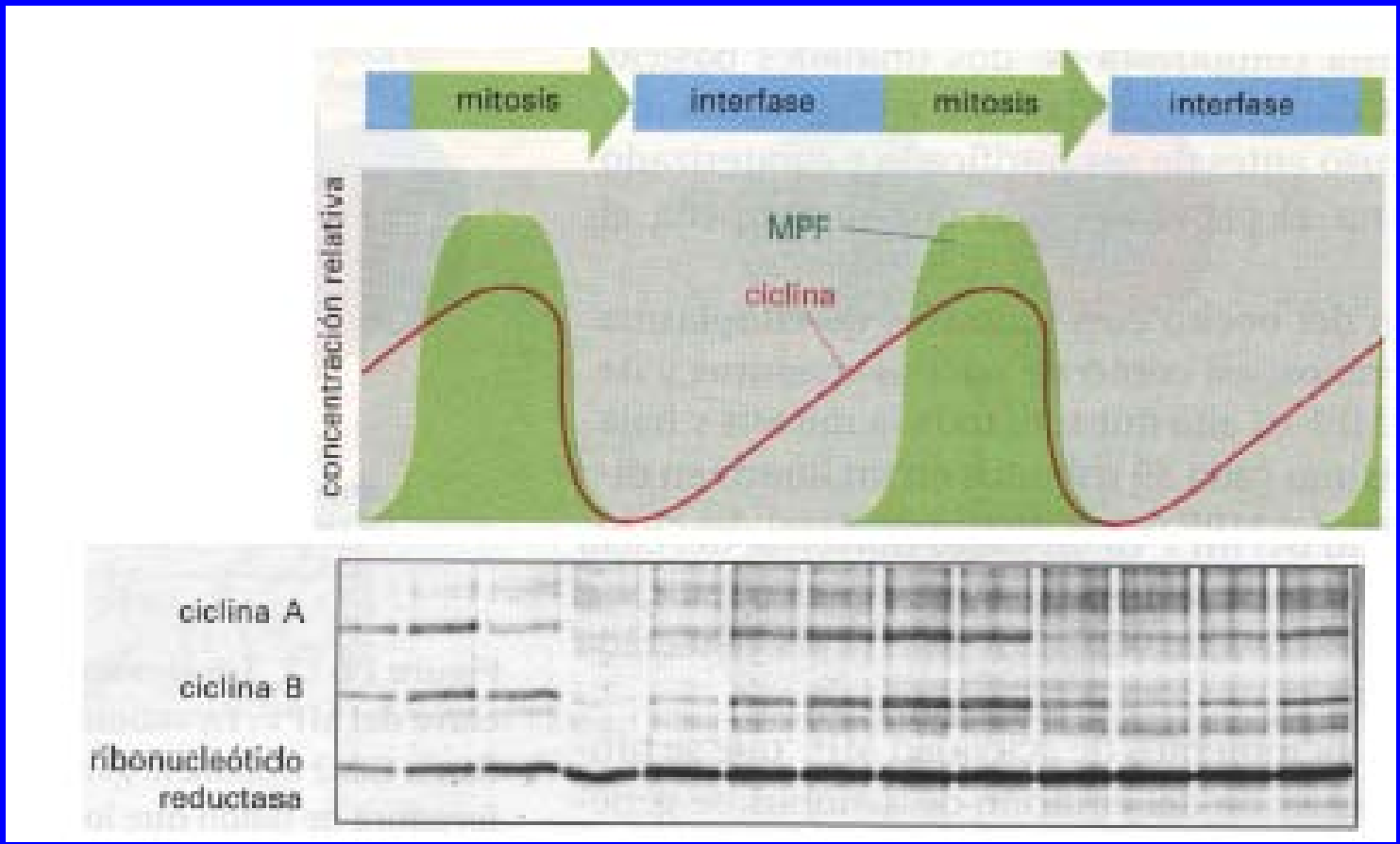
Lesiones geneticas:

1. Dominante: ganancia fx proto-oncogen >> oncogen
2. Recesiva: perdida fx gen supresor tumoral (oncosupresor, anti-oncogen)

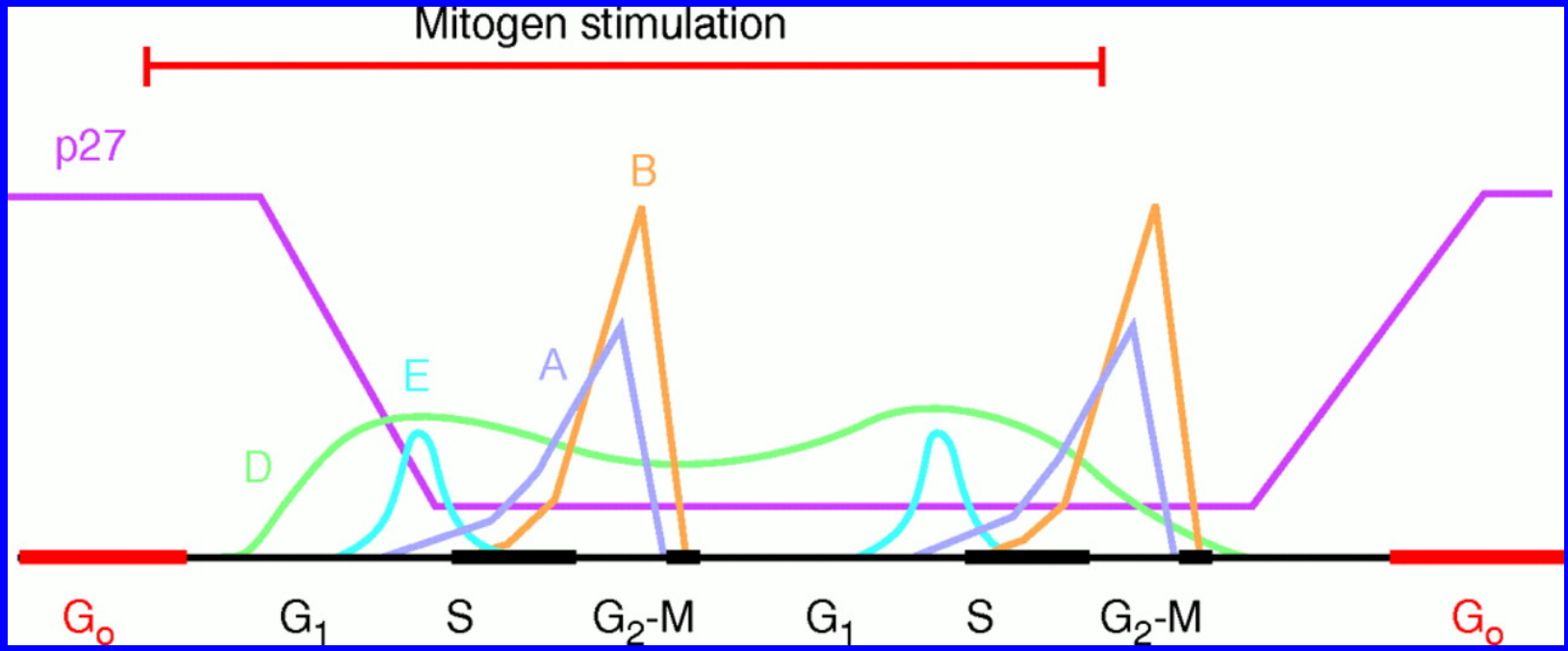
Ciclo celular eucariota



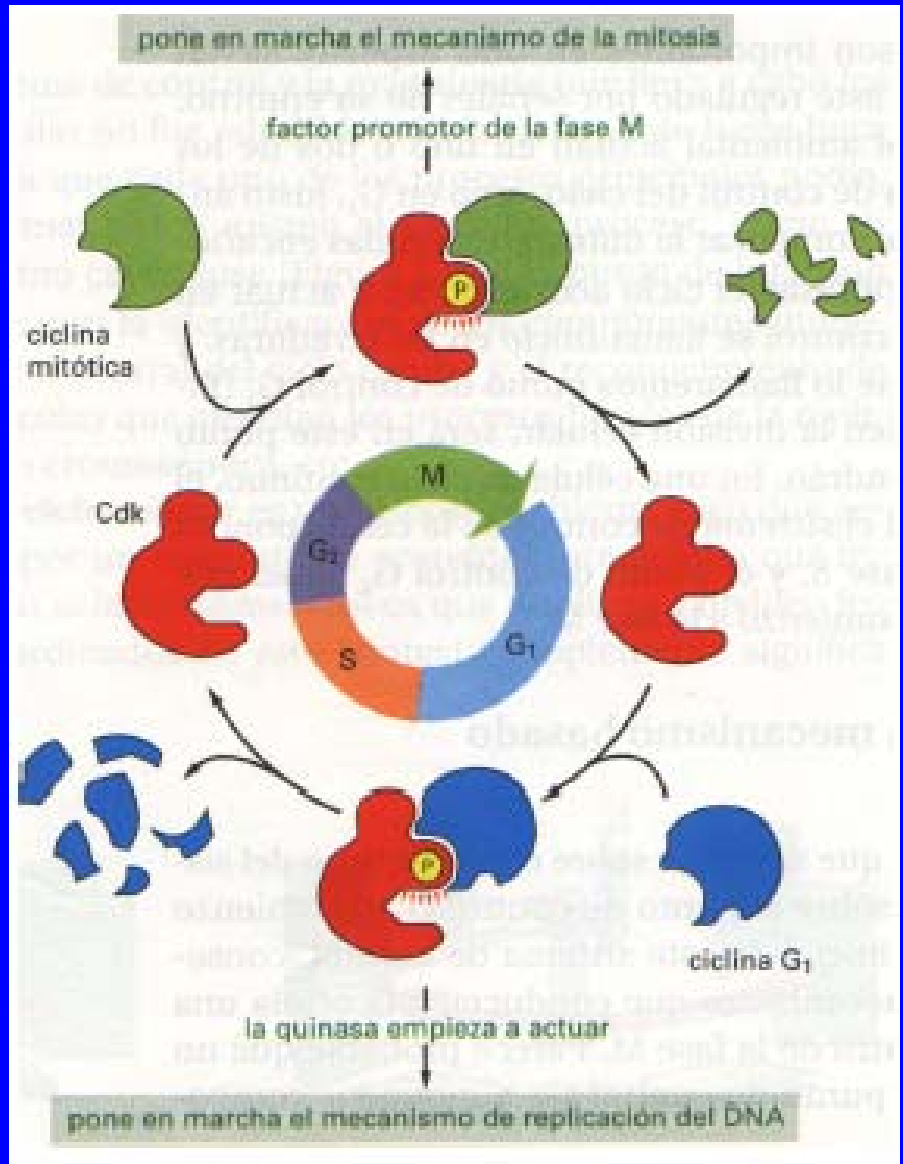
Existencia de “ciclinas” como componente del MPF



Ciclo celular humano



Ciclo celular y CDKs + Ciclinas



MPF: Ciclina+ Quinasa

-Componente efector: CDK

-Componente regulador: Ciclina

→ CDK+Ciclina:Quinasa activa

CDK:Quinasa inactiva

-Interruptor encendido: CDK+ Ciclina

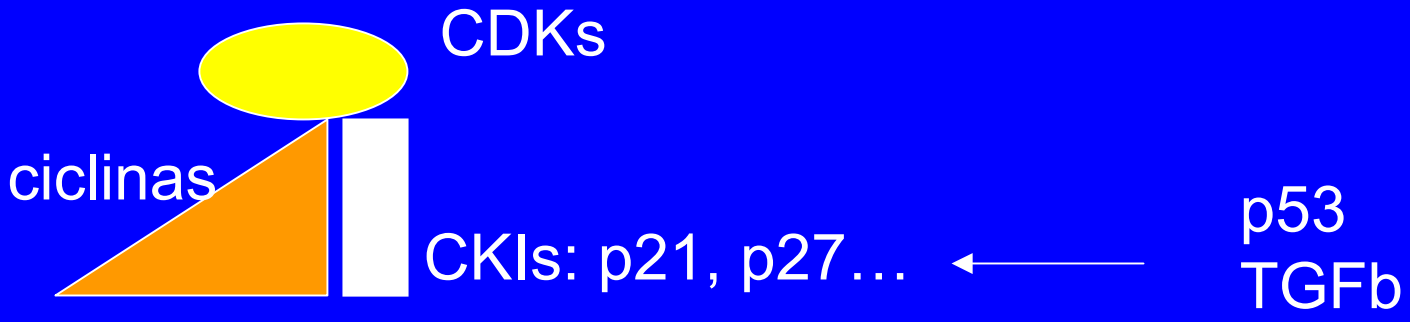
-Interruptor apagado: CDK (Ciclina degradada: ubiquinación)

-CDK fosforila en mitosis: -Lamina nuclear

-Histona H1

-Micotúbulos

Checkpoints: - G1-S: integridad genoma? nutrientes?
- G2-M: sintesis DNA completa?



Los propios genes de la maquinaria del ciclo pueden estar mutados en cancer:
-ciclina D1 (bcl1)
-CDK4
-CKIs: p16, p27,

ONCOGENES:

-factores crecimiento (ligandos y receptores):

TGFa, erbB, kit…

-Transduccion:

ras, prot.G…

- factores transcripcion:

myc, jun, fos…

Alteraciones representativas en neoplasias:

1. Cambios de base: *ras*
2. Amplificacion: *erbB2, myc*
3. Translocacion:
   - alt. regulacion (*myc)* t(8;14), Burkitt
   - proteina de fusion (*abl-bcr*) t(9;22), LMC
4. Insercion retrovirus

GENES SUPRESORES TUMORALES:

-factores crecimiento y prot.membrana:

TGFb, adherinas…

-transduccion:

NF1 (GAP), APC…

- factores transcripcion:

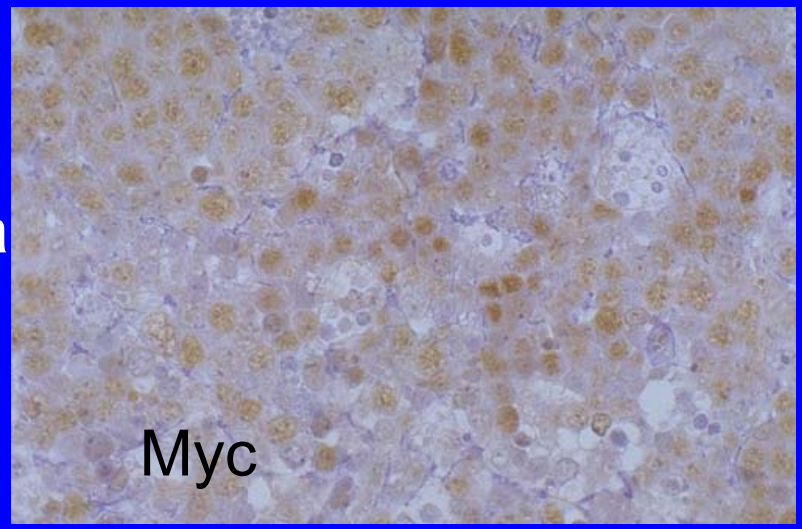
p53, Rb…

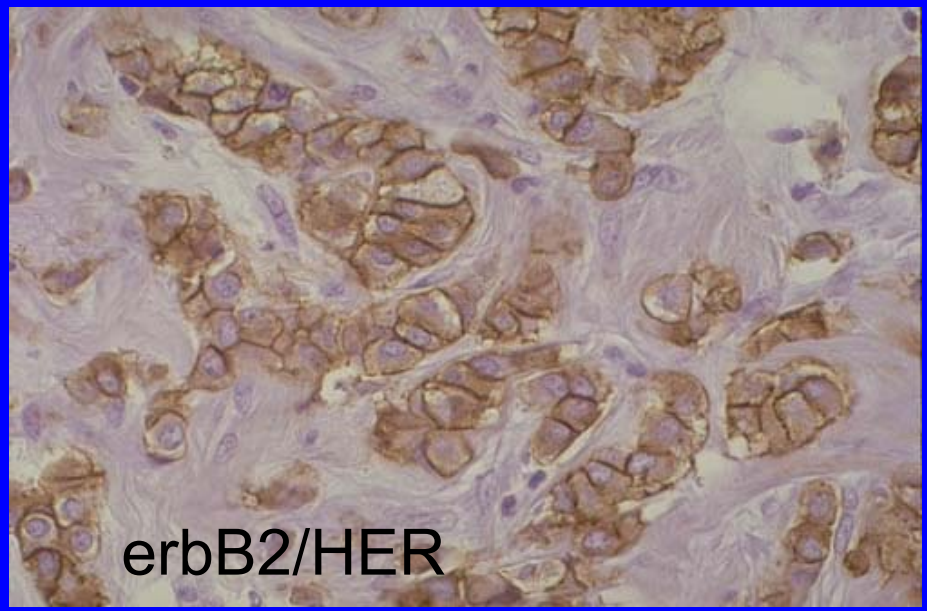
Alteraciones representativas en neoplasias:

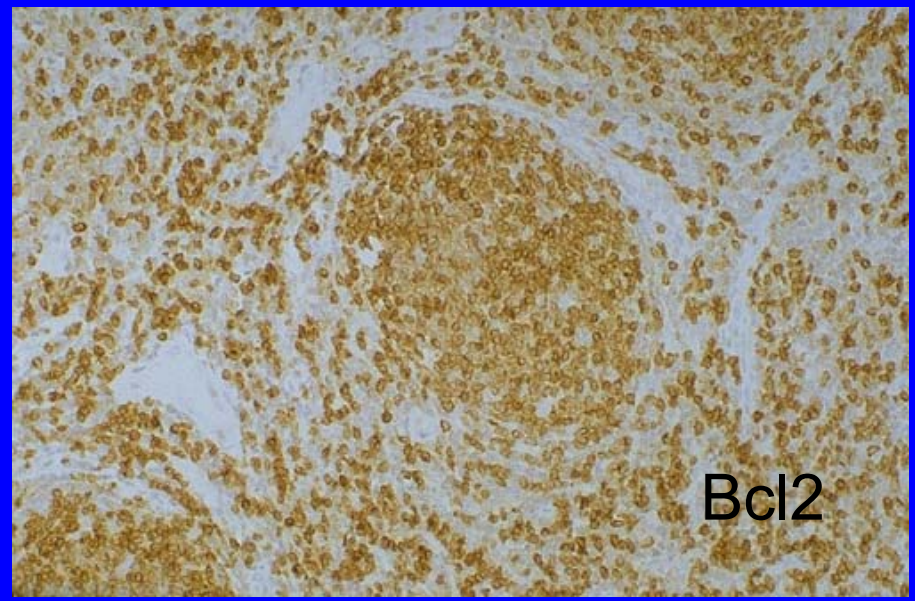
1. Cambios de base: *p53*
2. Deleccion: *DCC*
3. Interaccion DNA virus: *HPV* (E6-p53, E7-Rb)

Utilidad DxMolecular Cancer

1. Diagnostico: *abl-bcr* en LMC
   Bcl2 en linfomas
2. Pronostico: *myc* en neuroblastoma
   erbB2 en Ca.mama
   p53 en astrocitomas
3. Tratamiento (…)



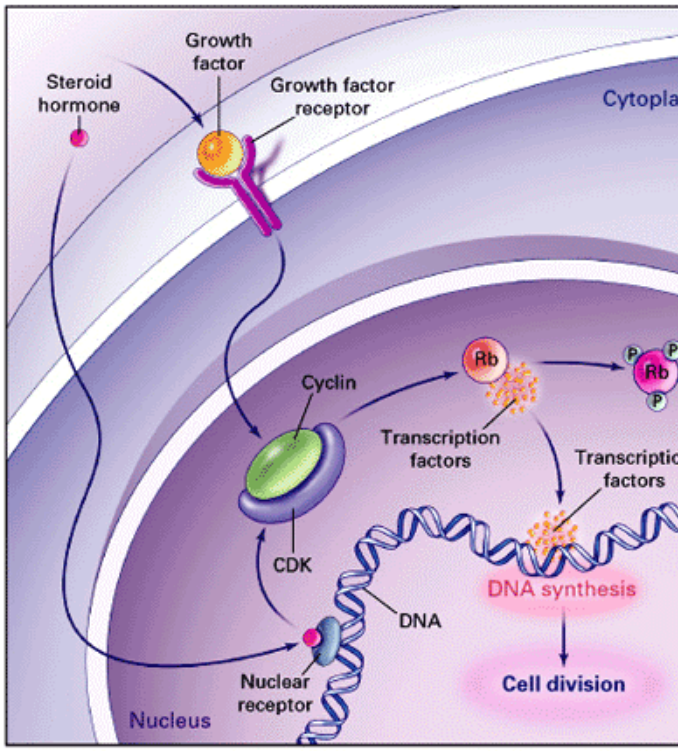


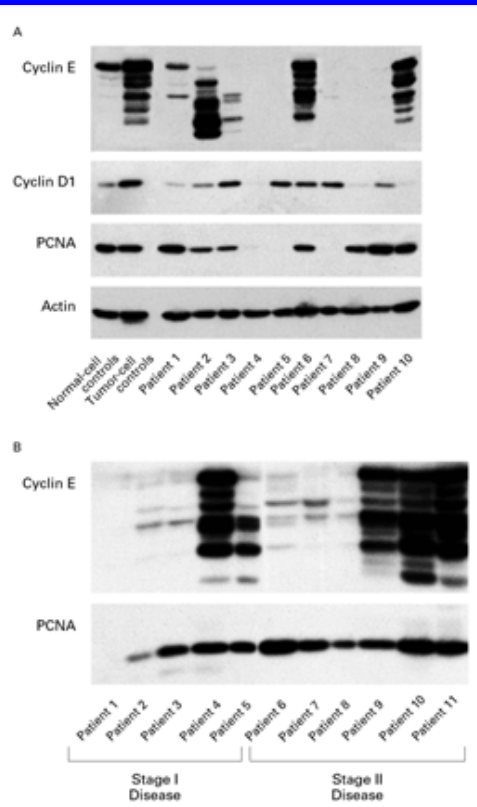


...descubriendose nuevos marcadores pronosticos...

NEJM 347:1566, 2002

Ciclina E y pronostico Ca.mama





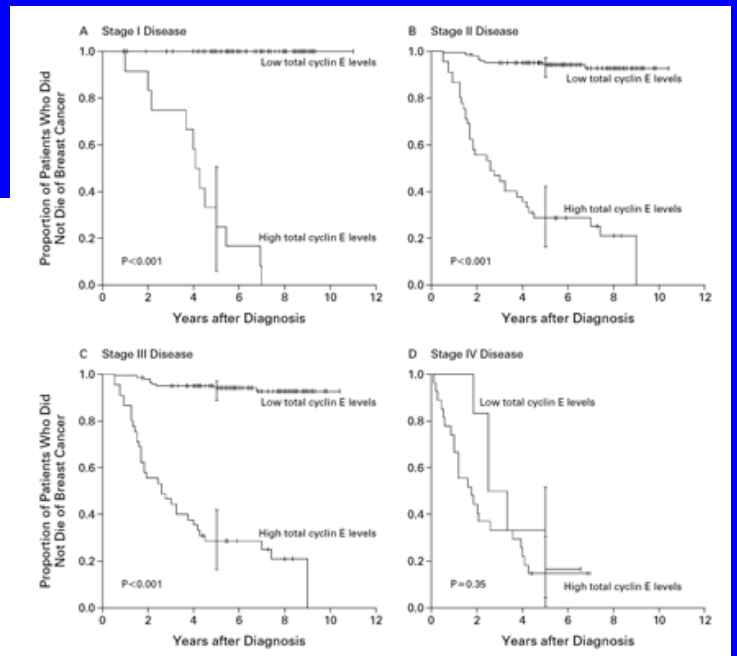


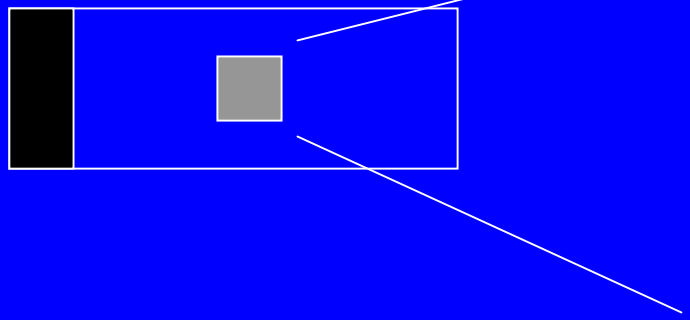
TABLE 2. INDEPENDENT FACTORS PREDICTIVE OF DEATH FROM BREAST CANCER AND DEATH FROM ANY CAUSE.*

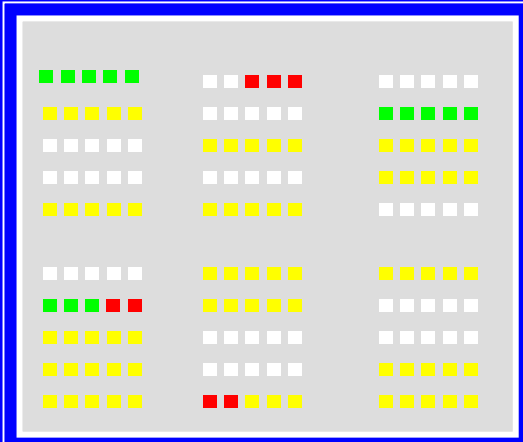
FACTOR	DEATH FROM BREAST CANCER		DEATH FROM ANY CAUSE	
	HAZARD RATIO (95% CI)	P VALUE	HAZARD RATIO (95% CI)	P VALUE
High level of low-molecular-weight cyclin E	2.1 (1.1–4.0)	0.02	2.2 (1.2–4.2)	0.01
High total cyclin E level	13.3 (5.8–30.2)	<0.001	4.3 (2.2–8.4)	<0.001
Positive nodes	1.8 (1.2–2.8)	0.007	1.5 (1.1–2.2)	0.02
Stage IIIB–IV disease	1.7 (1.1–2.5)	0.01	1.7 (1.2–2.5)	0.004
Negative estrogen-receptor status	1.8 (1.3–2.7)	0.001	1.6 (1.1–2.2)	0.006

*P values were derived from the Cox proportional-hazards model, with simultaneous inclusion of all factors shown. CI denotes confidence interval.

Analisis global de la expresion genica
- perfil de expresion genica del tumor -

DNA chip / matriz de DNA





Sondas moleculares identifican cada mRNA

tumor

sano

mRNA → **cDNA /fluorescente (verde)**

mRNA → **cDNA /fluorescente (rojo)**

Microscopio / scanner
análisis imagen

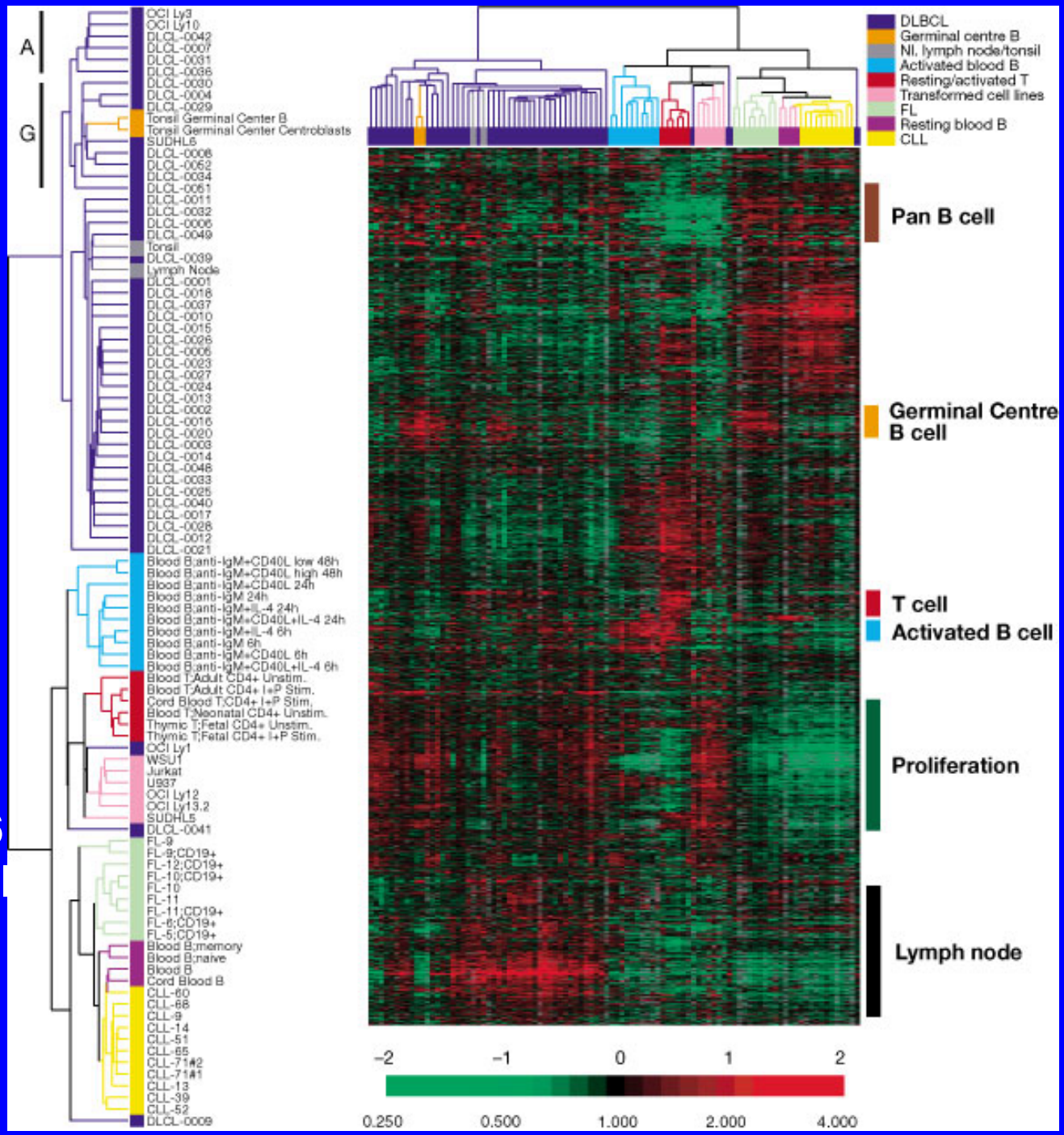
análisis datos → **correlación clínica**

Linfomas B

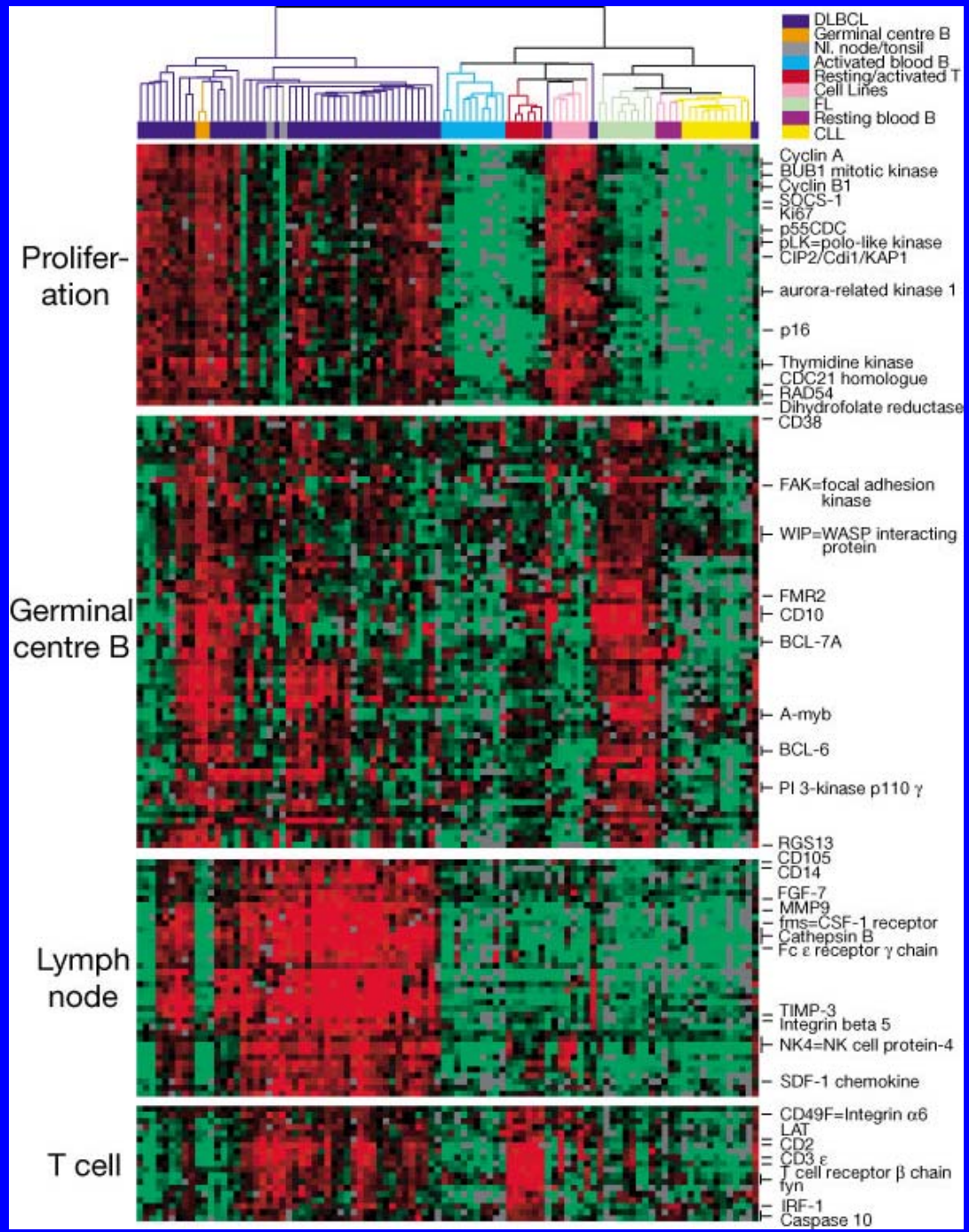
Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling
Alizadeh ...
Nature 403:503

'linfochip'
n=17,856
mayoría de genoteca de centro germinal
25% representados 2 o más veces

Cy5: experimental n= 96
-DLBCL, CLL, FL,normal
Cy3: control
-9 líneas linfoma
128 hibridaciones
1.8M mediciones



Linfochip en detalle



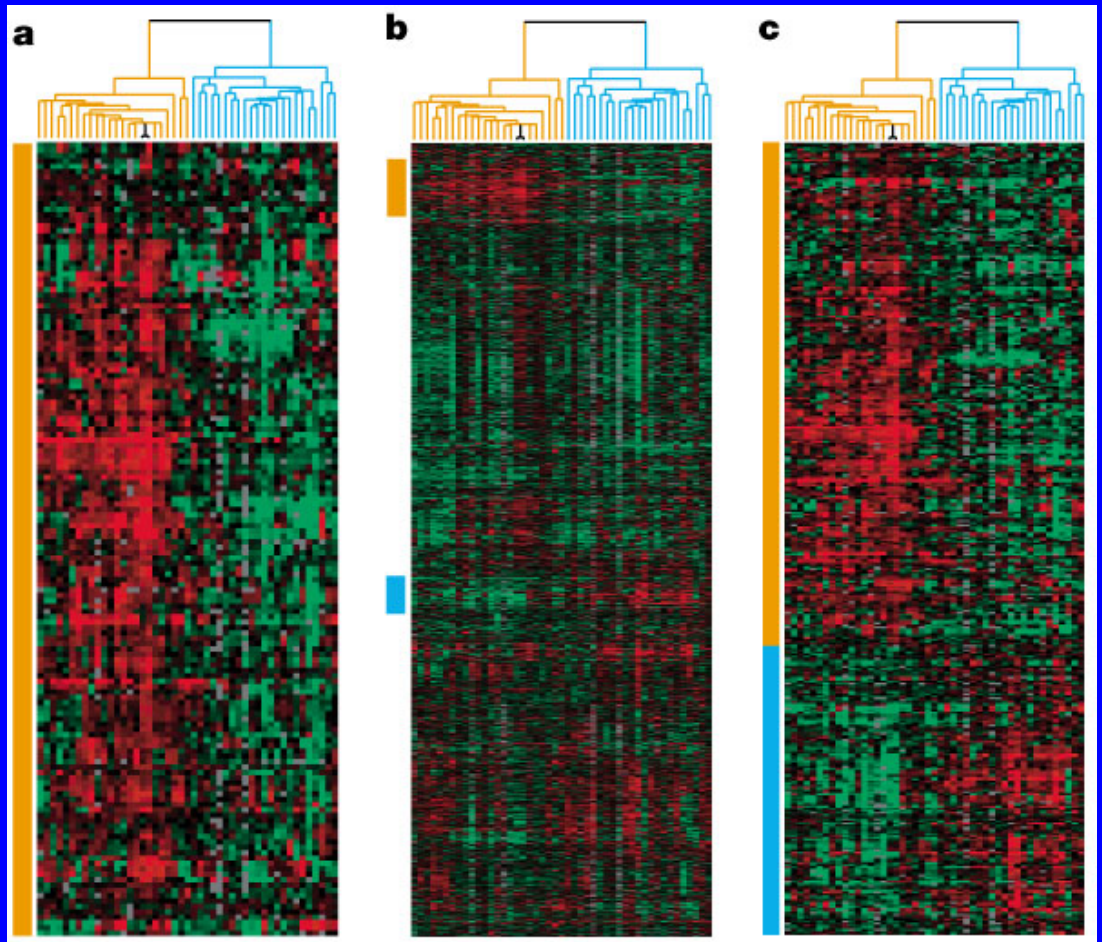
Subtipos de DLBCL según expresión

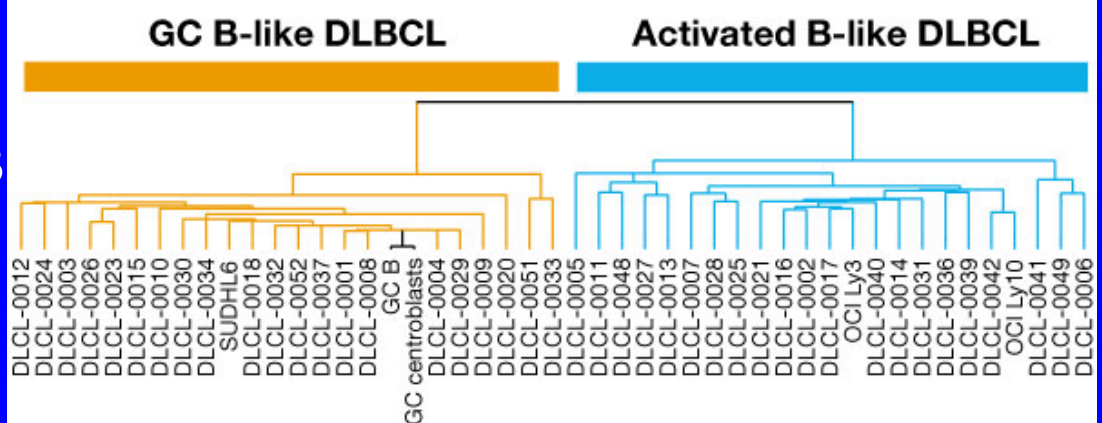
X: muestras de DLBCL
Y: genes:
Y1 (GC)
Y2 (todos)
Y3 (GC+act.B)

a) agrupa según expresión de genes centro germinales (GC)
X= f (Y1)

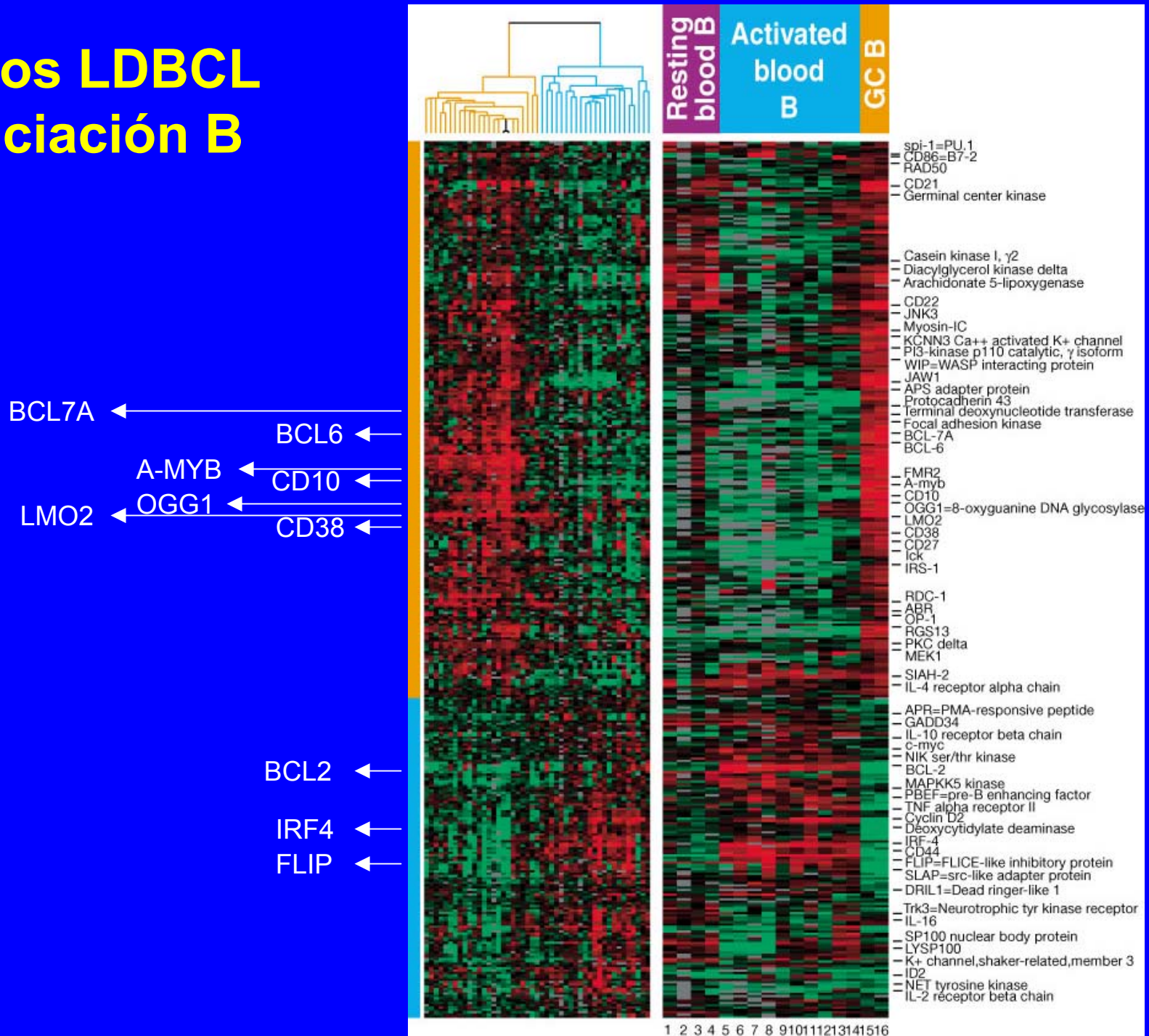
b) mantén posición de muestras y agrupa todos los genes
Y2= f(X)

c) mantén posición de muestras y agrupa los genes de GC y act.B
Y3= f(X)

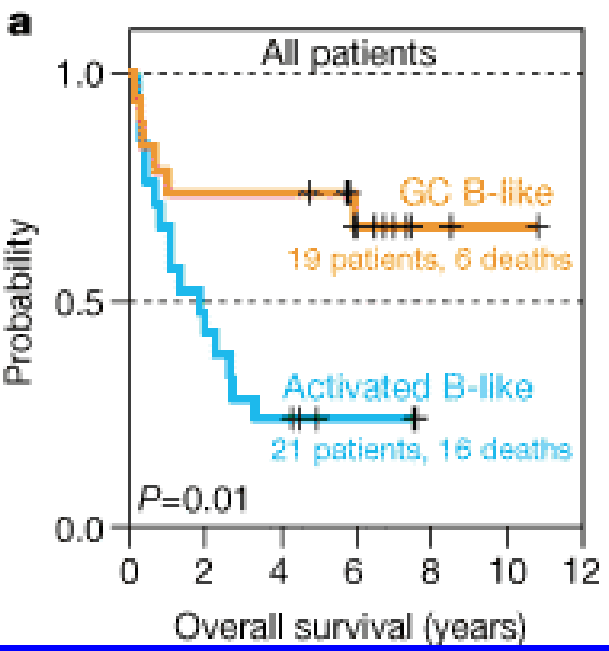


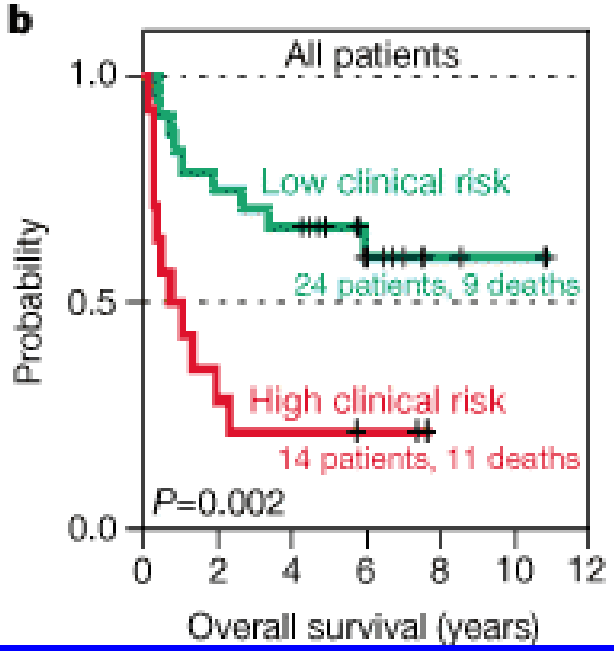


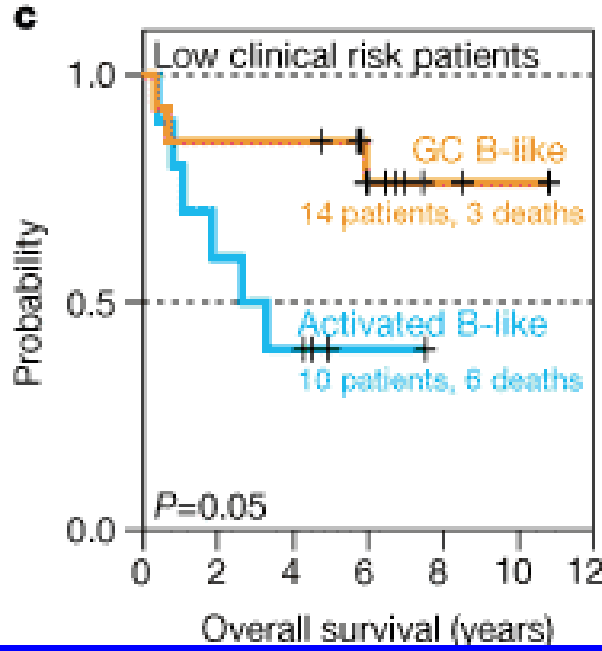
Subgrupos LDBCL y diferenciación B



Subgrupos pronósticos DLBCL / perfil de expresión génica







Según:... expresión génica

...Int. Prognostic Index

...expresión génica sólo los de IPI 0-2

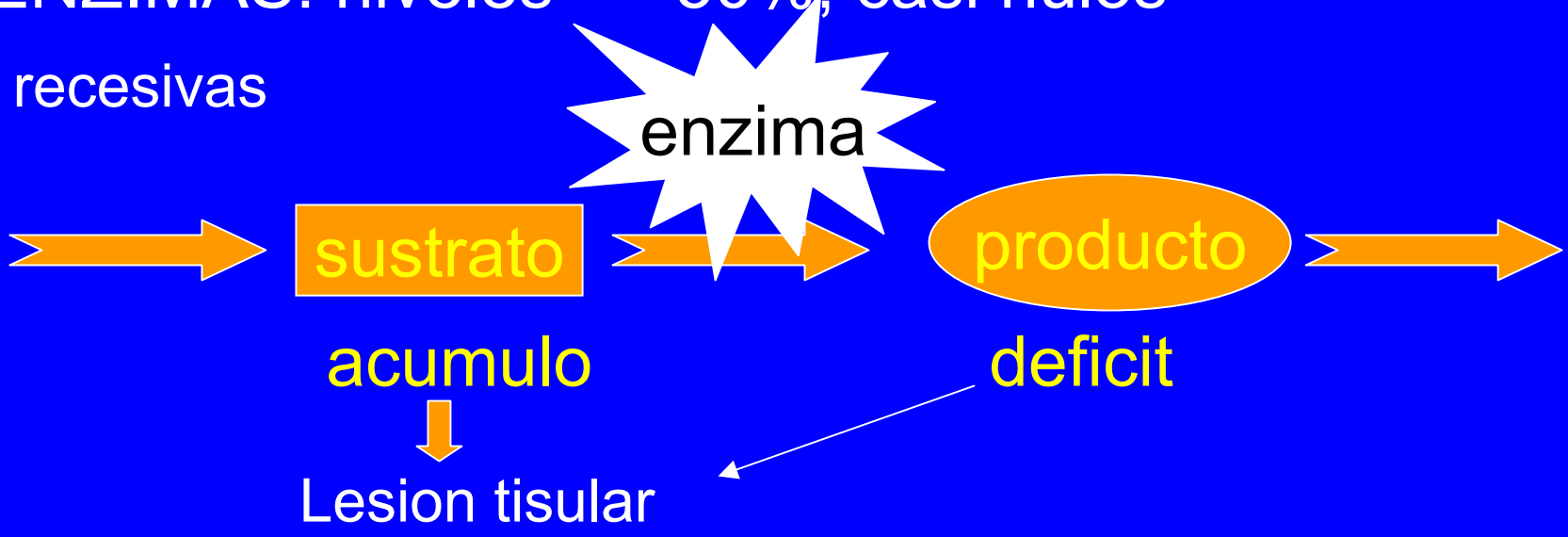
Deficits enzimaticos

-perdida Fx.

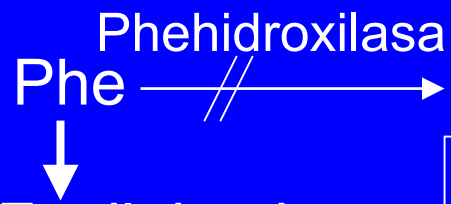
tipos mutaciones

ENZIMAS: niveles << 50%, casi nulos

recesivas



FENILCETONURIA:



Desmielinizacion
Degeneracion SNC

G/A i12spl: < 1%act.

G/A R261Q: > 5%act.
HiperPhe benigna

ALCAPTONURIA

Phe….Tyr….Homogentisico //. …. …. Fumarico

↓ HGO (ej. P230S, V300G….)

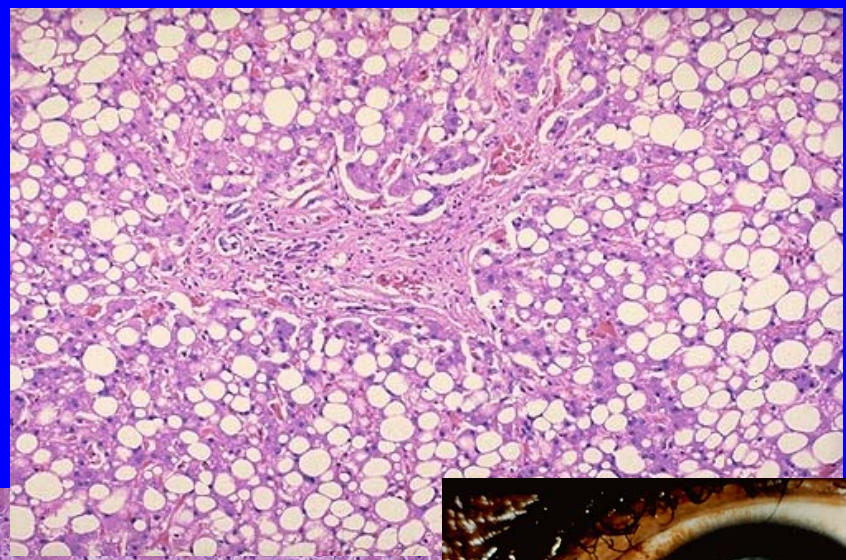
-orina: oxidacion… pigmento negro
-matriz conjuntiva: pigmentacion oscura mucosas, esclera
-cartilago: pigmentacion, inflamacion, degeneracion: OCRONOSIS

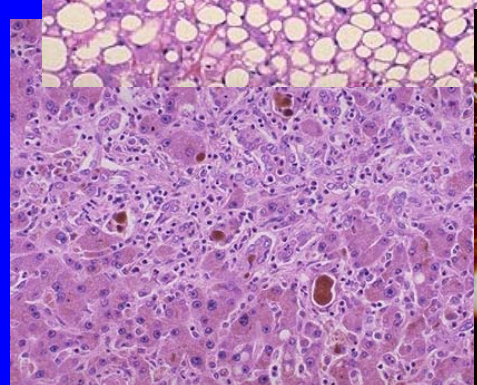
GALACTOSEMIA

Gal…Gal-1-P //..UDP-Gal

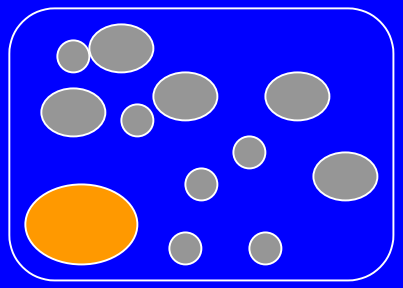
↓ GALT

Higado: esteatosis,
colestasis, fibrosis, cirrosis
SNC: gliosis, edema
Cataratas





Enf. Acumulo lisosomal



Deficit de algun enzima del lisosoma necesario para degradacion / reciclado de componentes celulares….. Acumulo progresivo de sustrato

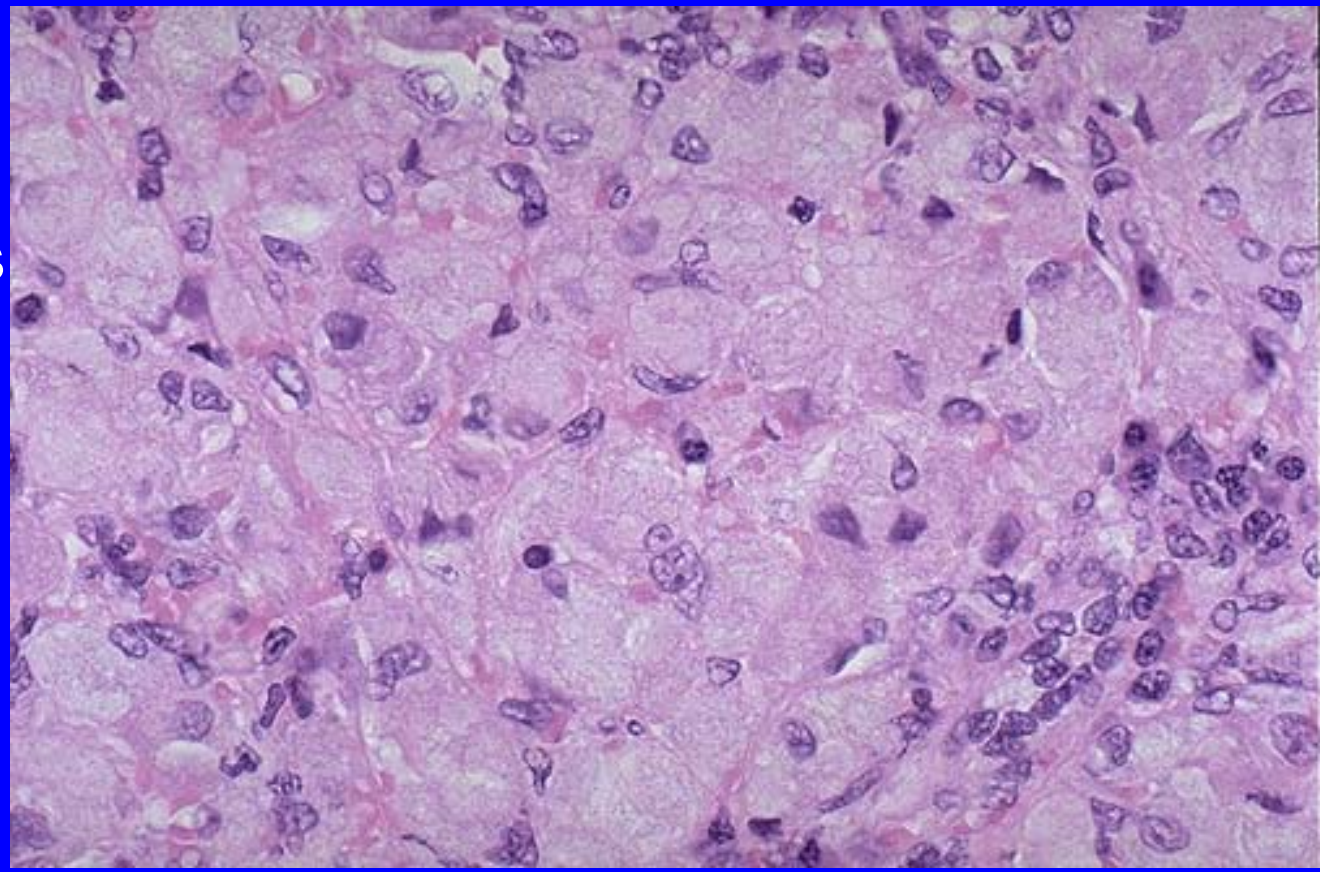
1. Esfingolipidosis: - def. Esfingomielinasa (Niemann-Pick)
   - def. Glucocerebrosidasa (Gaucher)
   - def. Arilsulfatasa A (leucodistr.metacrom.)
   - def. Galactosidasa A (Fabry)
   - def. Gangliosidogalactosidasa (gang.GM1)
   - def. Hexosaminidasa (GM2, Tay-Sachs)
2. Mucopolisacaridosis
3. Glicoproteinosis
4. Glucogenosis tipo 2 (Pompe)

Def. Glucocerebrosidasa (Gaucher)

-tipo I, CRONICA, NO-NEUROPATICA (99%): - hepatomegalia
- esplenomegalia

-tipo II, infantil, neuropatica: letal < 1a., SNC
-tipo III, juvenil

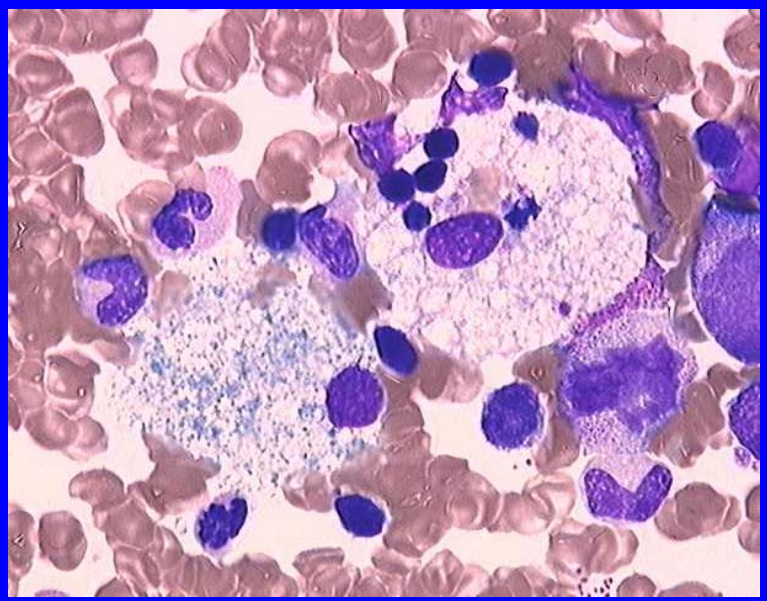
Cels. Gaucher:
Macrofagos grandes (hasta 100um)
"papel arrugado"
Higado, bazo, ganglios linfaticos



Def. Esfingomielinasa (Niemann-Pick)

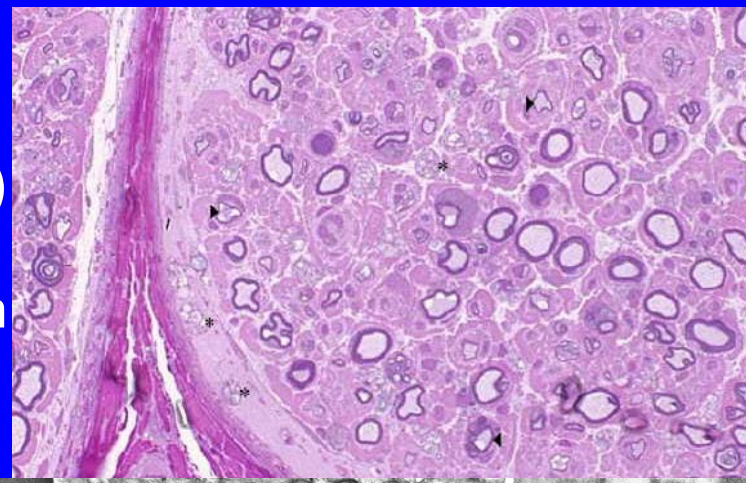
-tipo A, INFANTIL (80%): SNC, higado, bazo, letal (1-2a)
-tipo B, no neuropatica

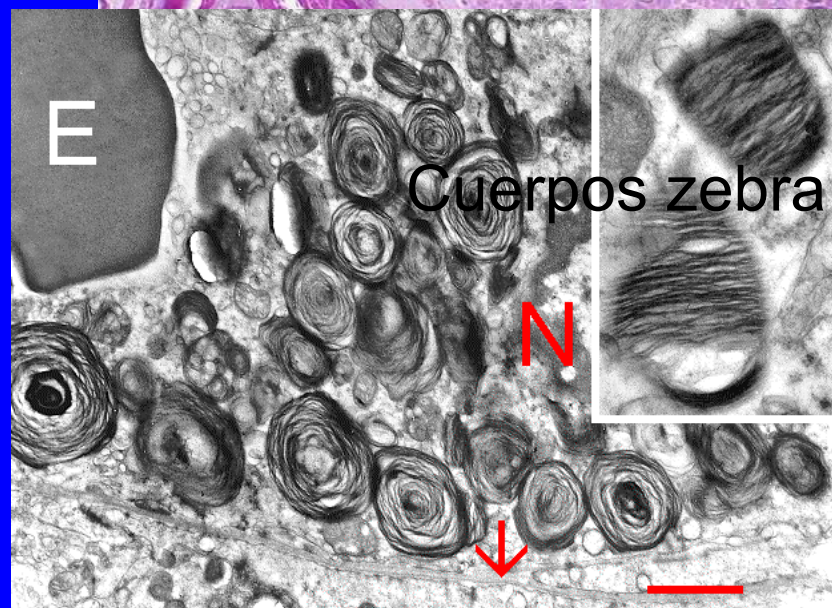
-tipo C (gen NPC1, metab.colesterol)



Macrofagos espumosos

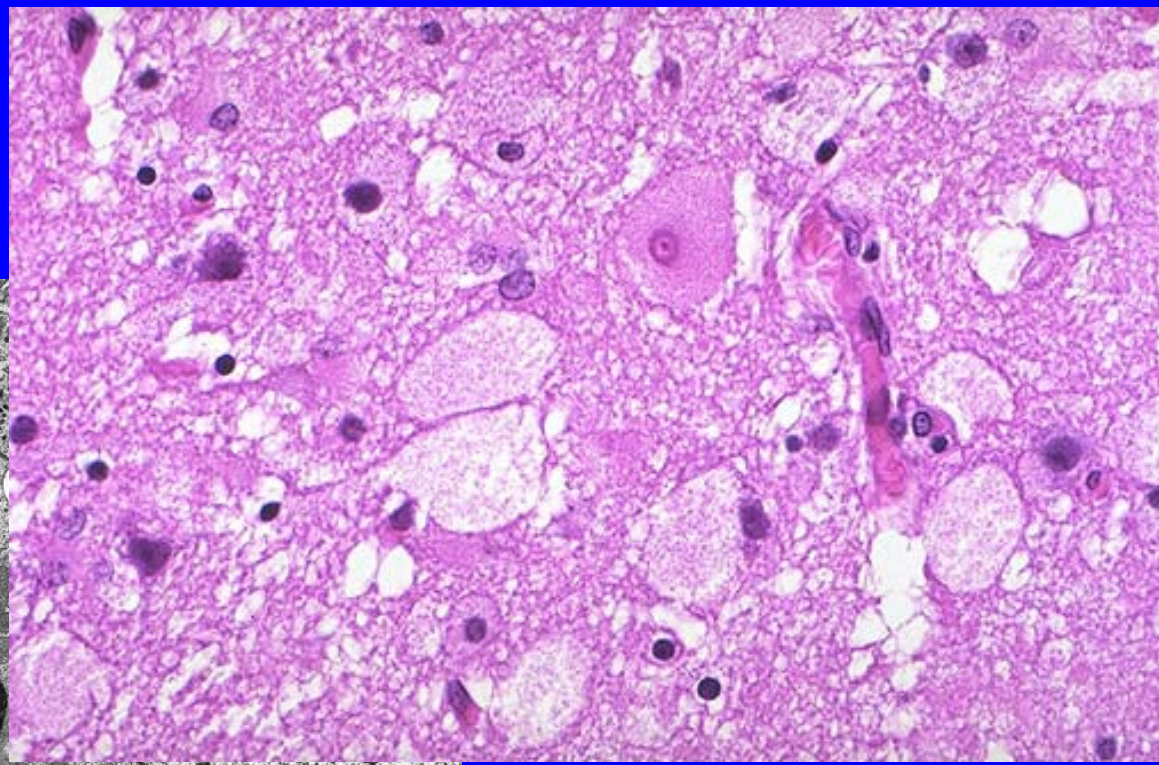
desmielinizacion

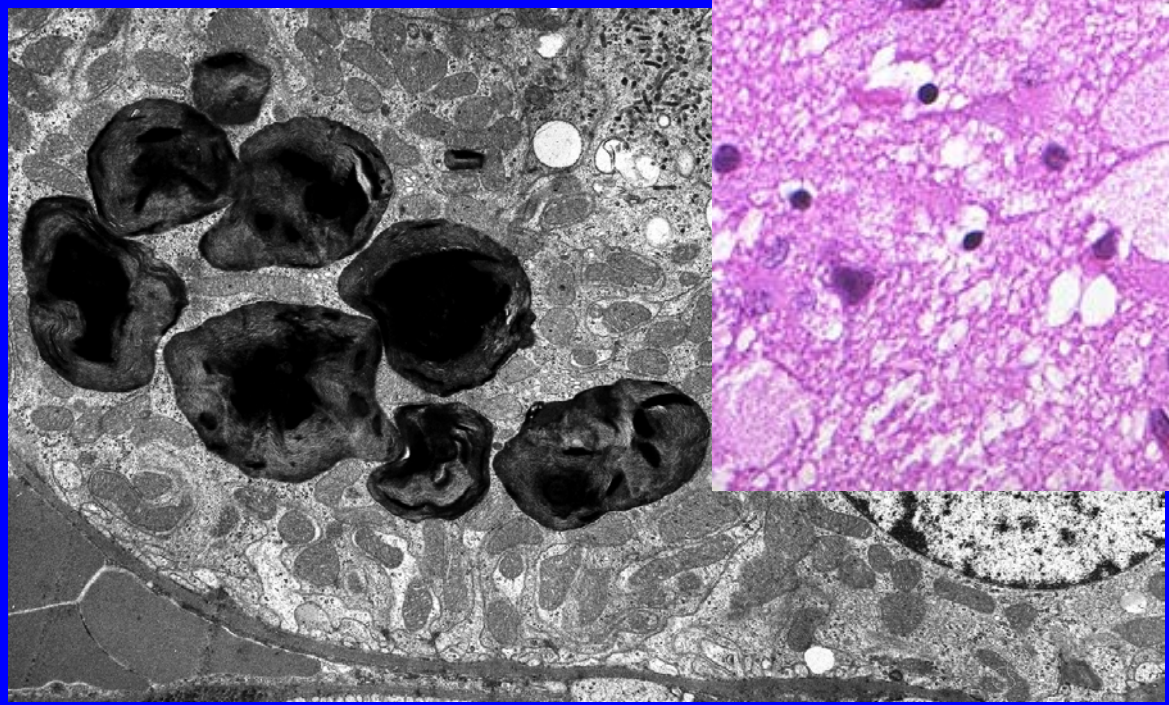




Def. Hexosaminidasa (Tay-Sachs)

-INFANTIL: afectacion neuronal, tambien macrofagos; (ins.4bp)
-Adulto: actividad residual (ej. G269S)



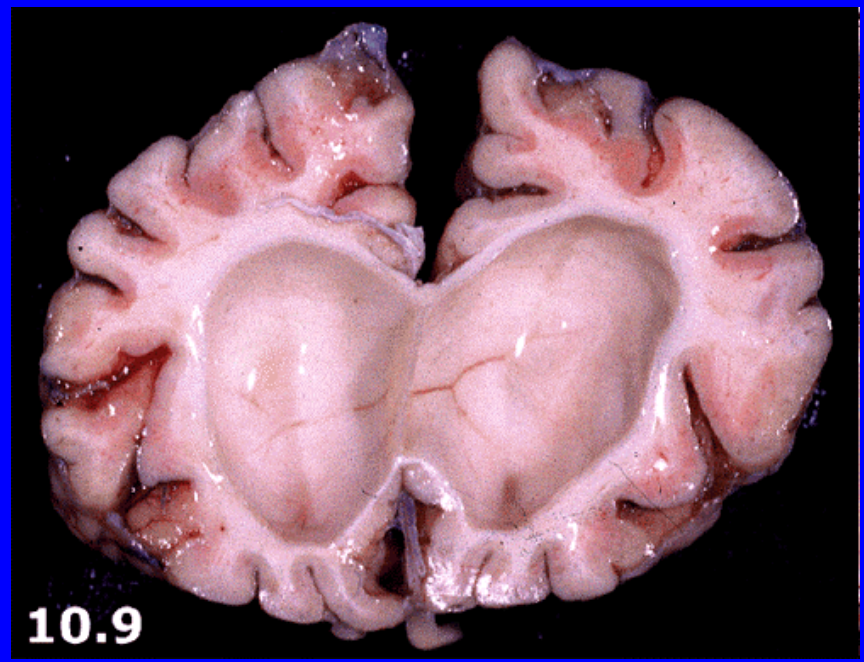


Mucopolisacaridosis

-glicosaminoglicanos (mucopolisacaridos: dermatan-S, heparan-S, keratan-S, a veces condroitin-S), higado, bazo, corazon, vasos, cornea, SNC… desfigurantes, retraso mental

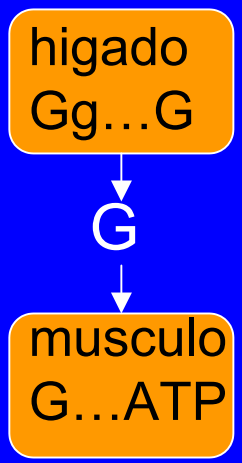
-degradacion jerarquizada: varios enzimas en la misma via:

- Iduronidasa A (autosomica, enf. Hurler)
- Iduronatosulfatasa (lig.X, enf Hunter)
- Heparansulfatasa, NAGlucosaminidasa…. (enf.Sanfilippo)



Glucogenosis

* solo la t.2 (maltasa acida, enf.Pompe) es lisosomal



- hepaticas: hepatomegalia, hipoglucemia
ej. def. Glucosa-6-Fosfatasa (t.1, von Gierke)

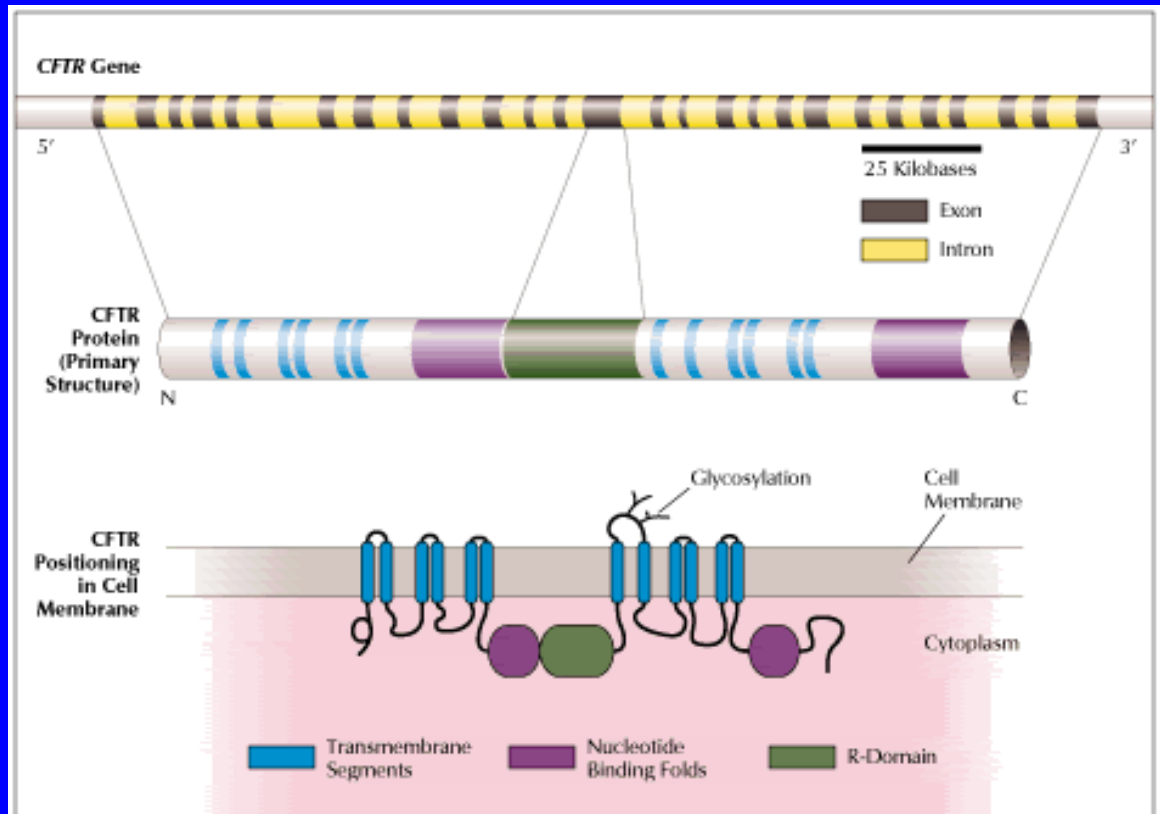
- musculares: miopatia, def.glucolisis
ej. def. Fosforilasa muscular (t.5, McArdle)

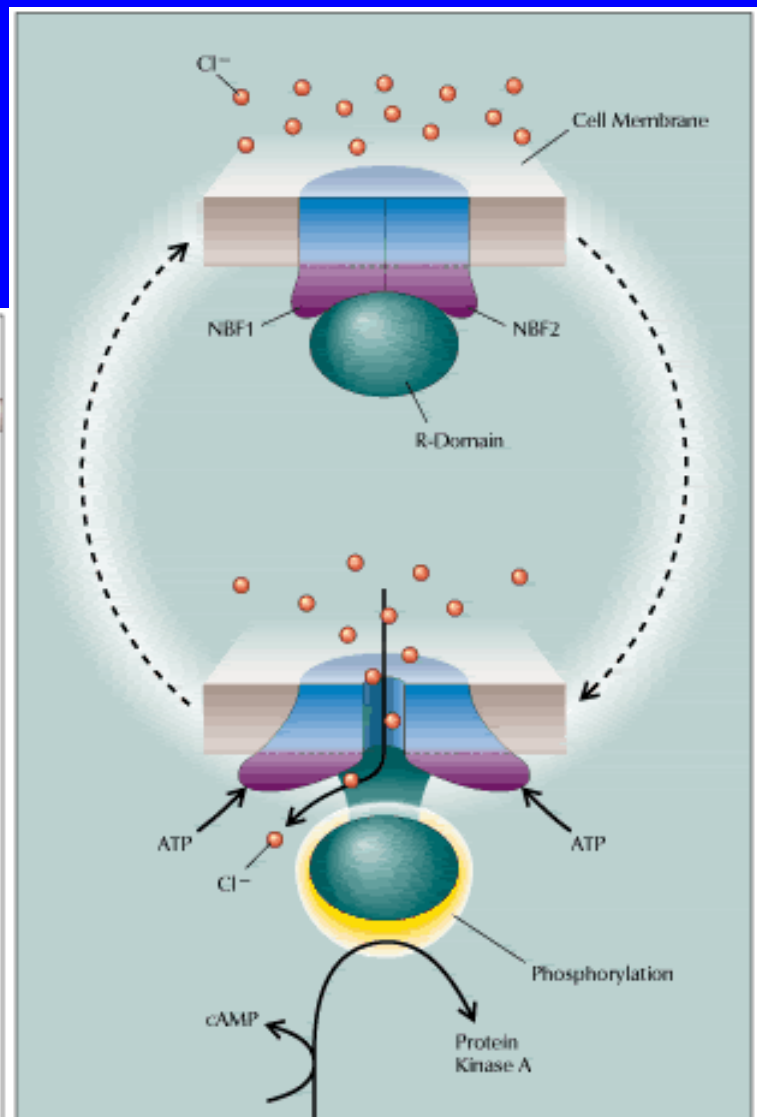
- generalizadas: higado, corazon, musculo
ej. def. Maltasa acida o glucosidasa lisosomal (t.2, Pompe)

Fibrosis Quistica

1:3,000

Trasportador Cl, reg. ATP
- glandulas epit.respitatorio
- conductos pancreaticos
- gl. sudoriparas



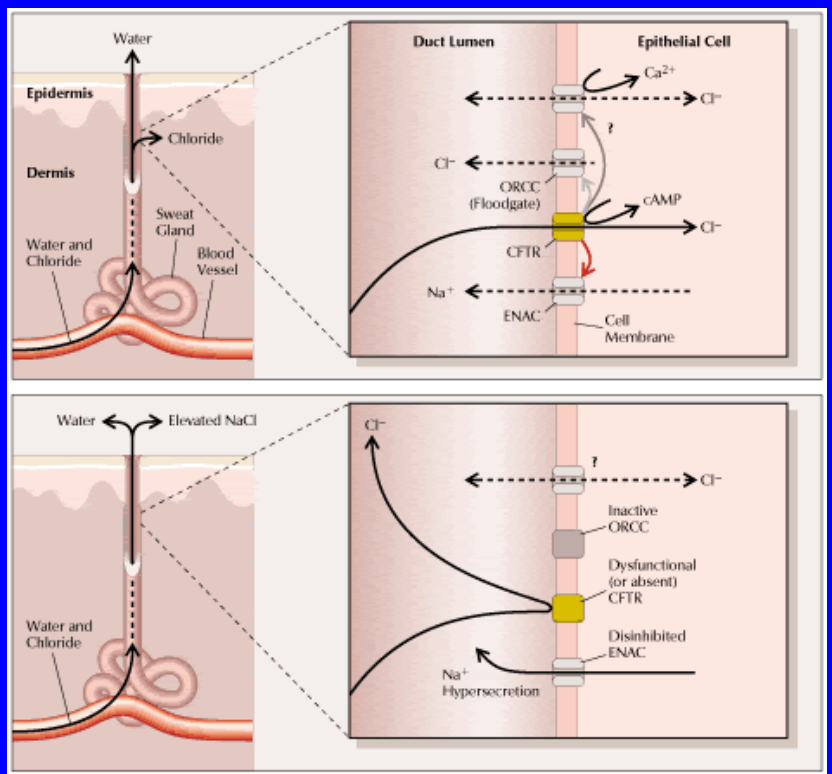


Segun el epitelio, CFTR media absorcion o excrecion

Gl.sudoripara:

(CFTR: absorcion)

FQ: sudor > Cl, Na



Gl.respiratoria, conductos:

(CFTR: excrecion)

FQ: no sale Cl, entra Na y H2O… deshidratacion de secreciones

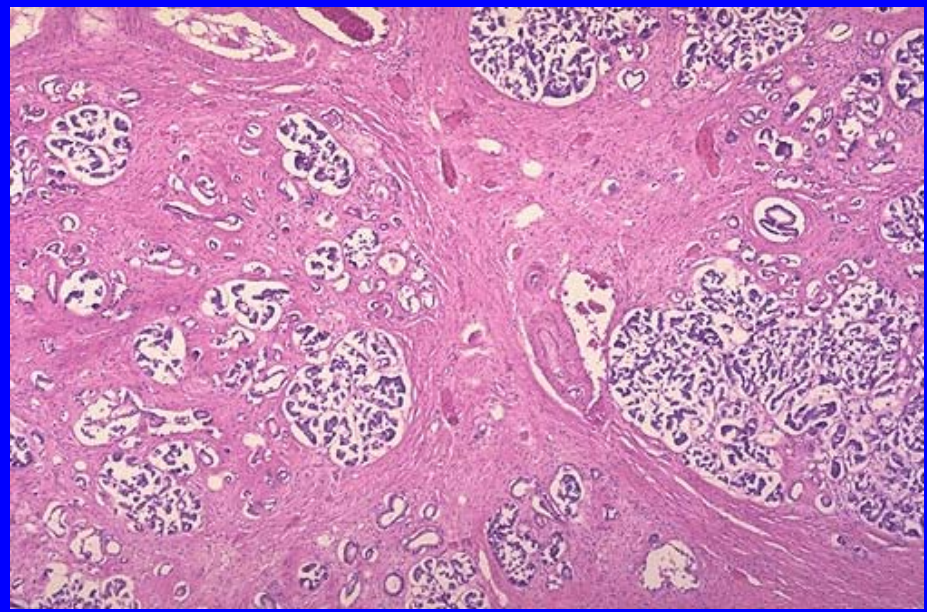
Mutaciones mas frecuentes:

-dF508 (0.7): defecto de procesado

-G542X (0.1): defecto de sintesis

Lesiones fibrosis quistica

-Pulmon: bronquitis (Pseudomona), bronquiectasias
-Pancreas: obstruccion conductos, fibrosis, quistes, atrofia
Atrofia v.deferente
-Ileo meconial y cirrosis biliar



Hemocromatosis

Depositos hierro en hepatocitos,… pancreas, corazon, endocrino, piel

- ferritina: proteina 24 subun., 480,000, nucleo de hidroxifosf.ferrico
- hemosiderina: producto de metabolismo ferritina en lisosomas, comp.var.

Exceso Fe libre > reacc.enzimaticas > radicales libres > lesion celular

-Secundaria: sobrecarga: eritropoyesis ineficaz, mielofibrosis…

-Hereditaria: A.R. ligamiento a HLA-A3, modificacion fenotipo: sexo gen HFE, familia MHC clase I

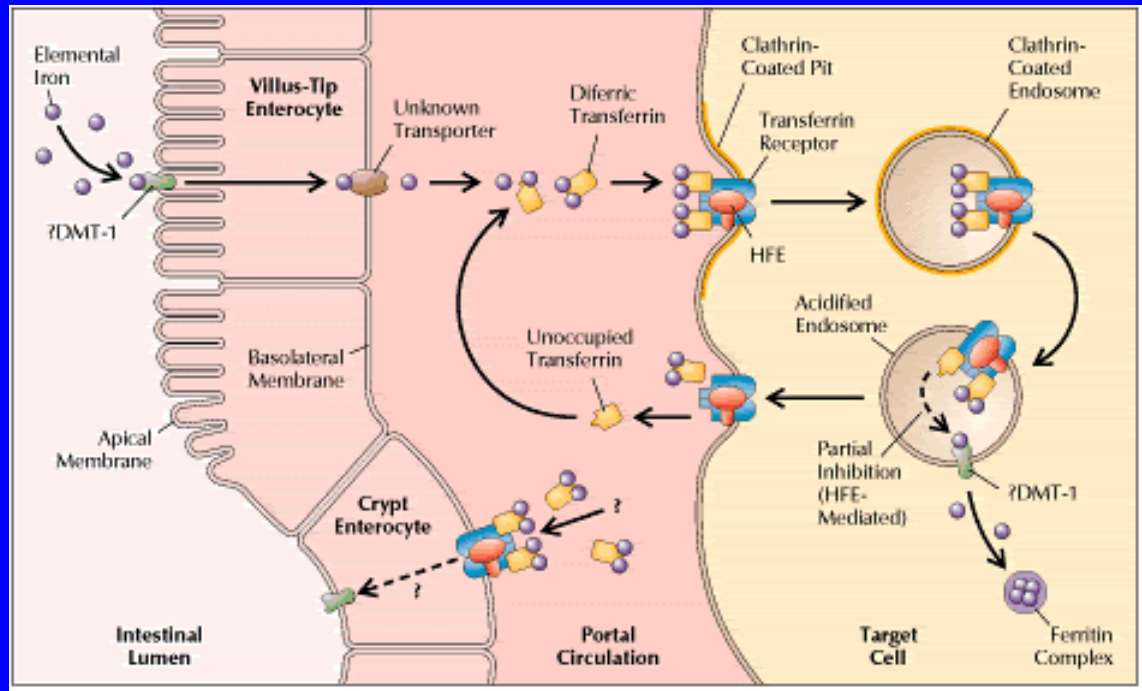
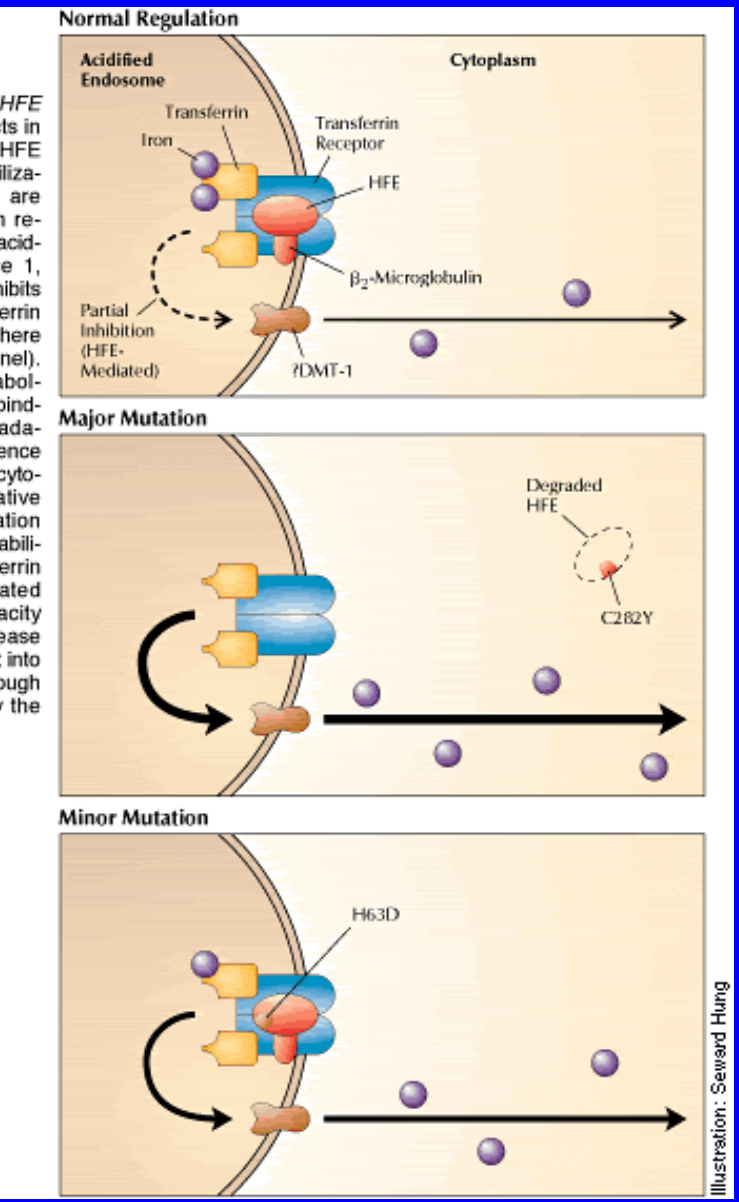


Figure 1. Emerging details of iron metabolism permit at least partial understanding of the function of the HFE protein—and of HFE's absence or dysfunction in causing hereditary hemochromatosis. From the intestinal lumen (left), dietary iron is transported into enterocytes, most likely by the newly described transporter DMT-1. From there iron enters the portal

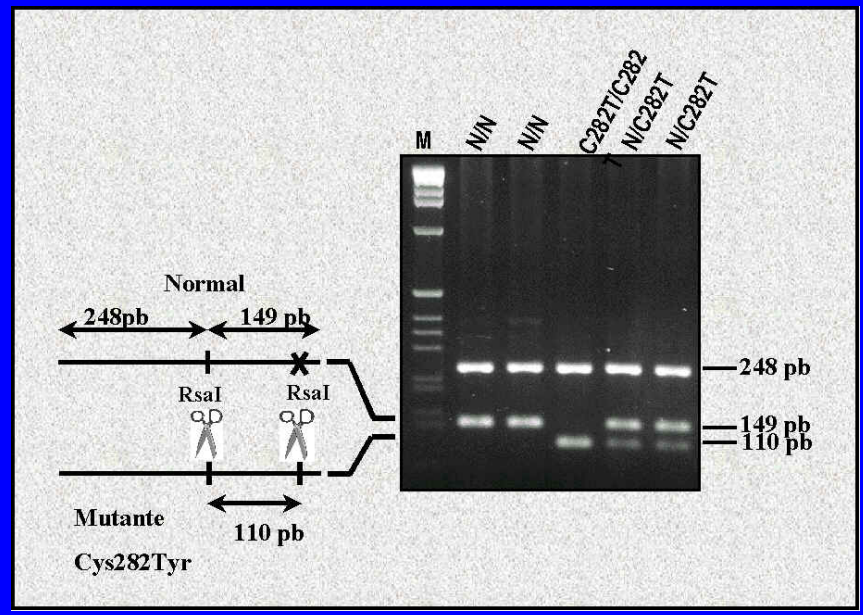
release of iron, so that an increased fraction of iron-bound transferrin recycles back out of the cell. In the absence of HFE, the cell may become iron-overloaded. A more primary problem may affect the intestinal lining. Here, HFE is hypothesized to act in undifferentiated crypt enterocytes (bottom left), the precursors of villus-tip enterocytes, so as to regulate uptake of

Mutaciones gen HFE

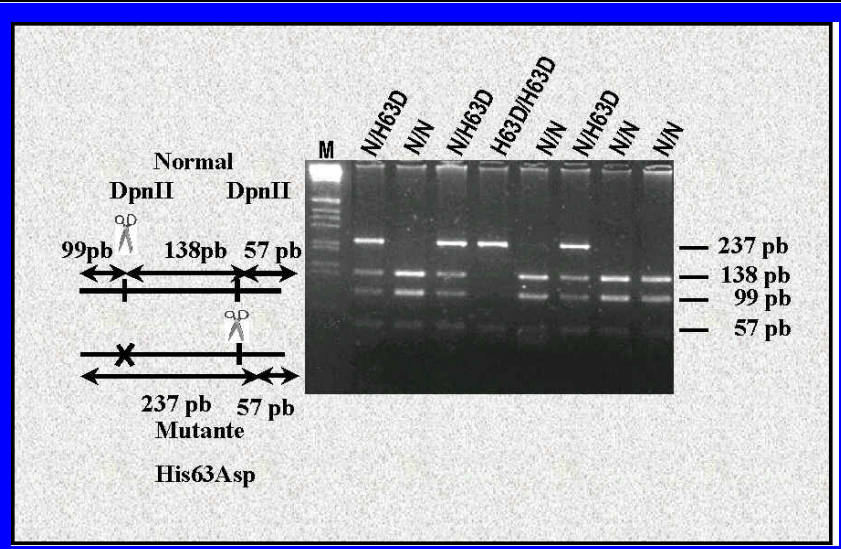
>85% alelos mutados son C282Y



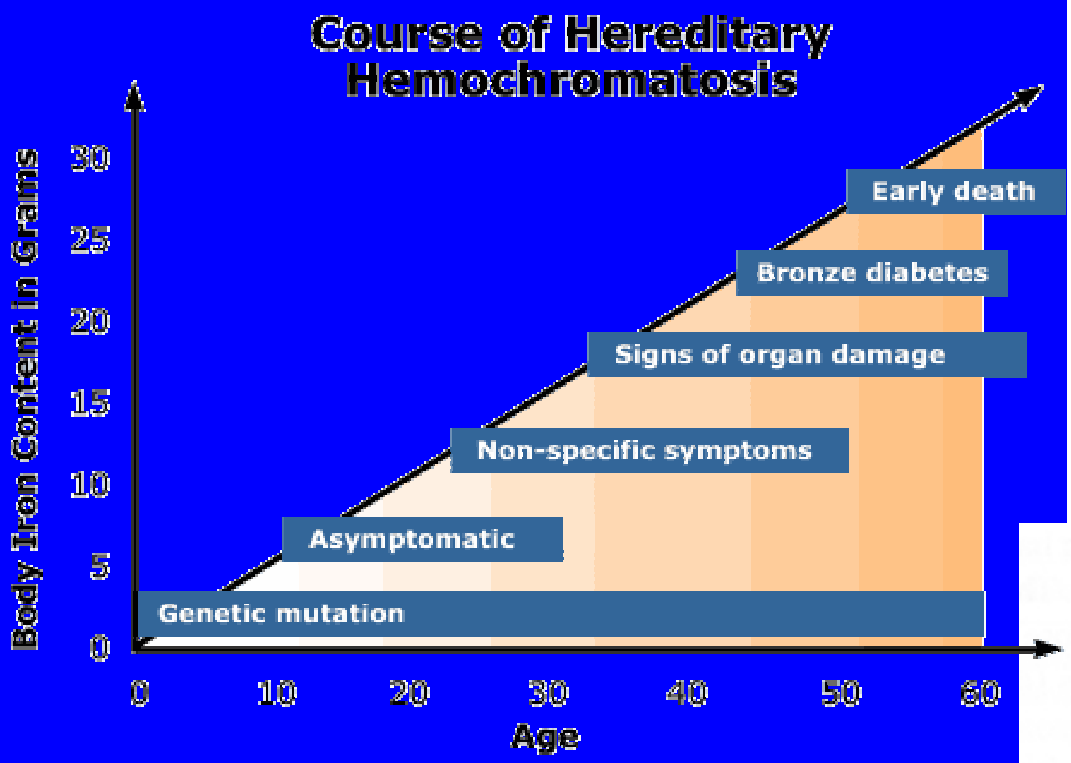
C282Y



H63D



Correlacion genotipo-fenotipo



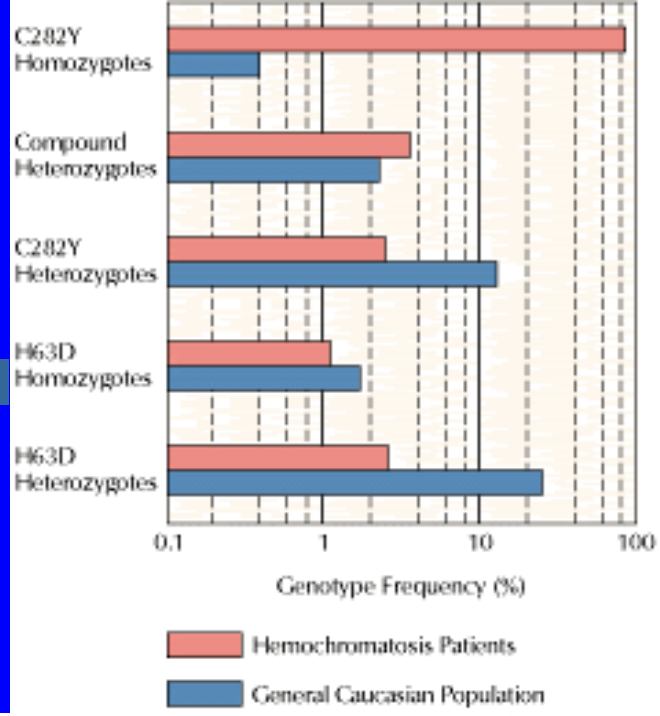
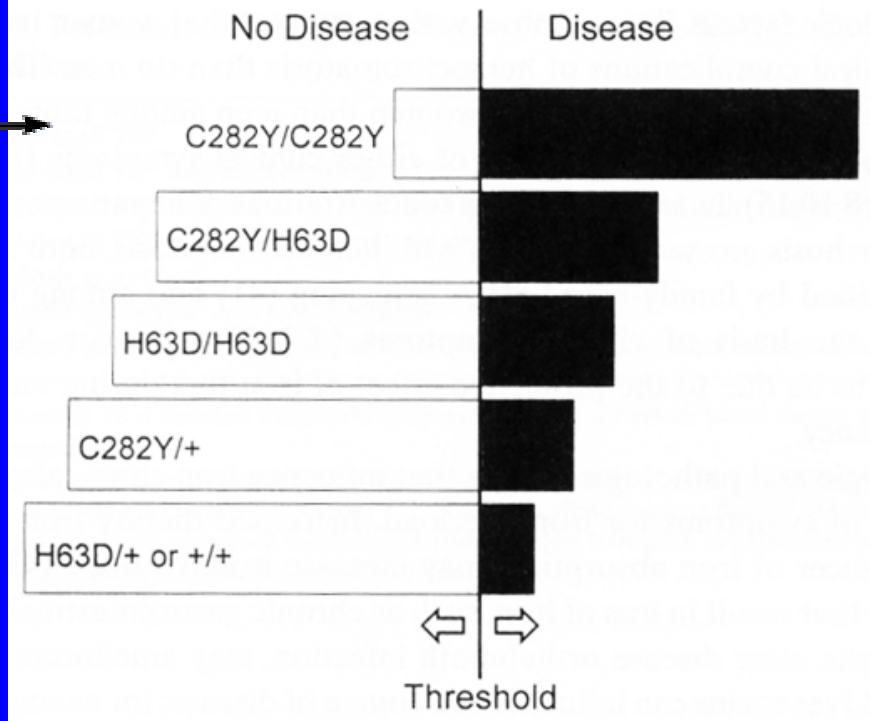
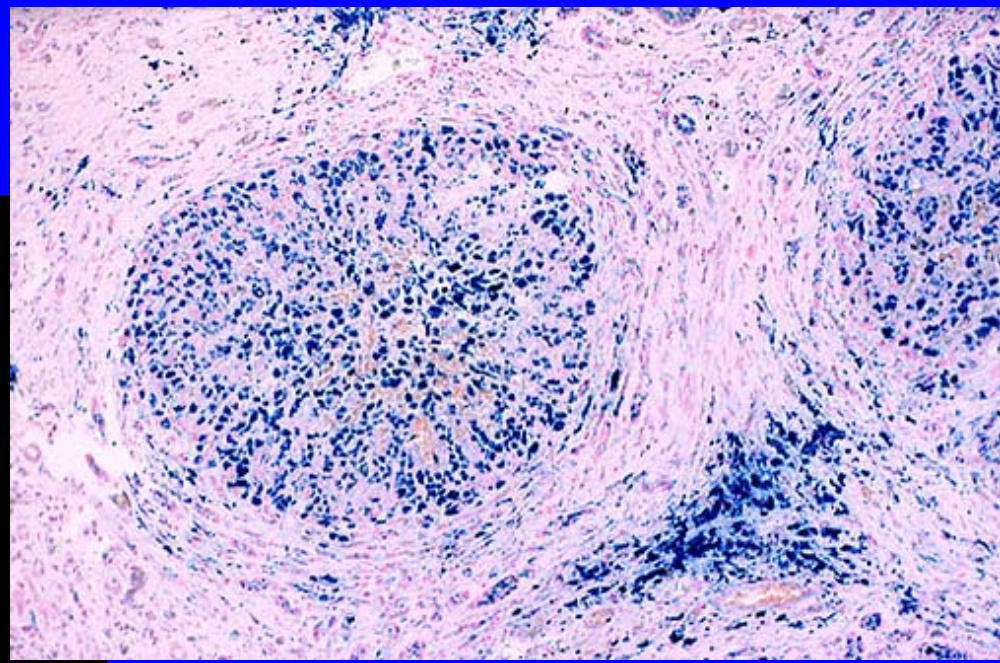


Figure 3. Ge
matosis pati
the contrast
tions in ca
major muta
90% of patie
4% of patien
ing one cop
tion H63D.
nearly as fre
minority of s
load develo
about equal
this genotyp
0.2%. Heter
is extremely
iron overloa
ulation carr
than 20% a
on a logarith
several surv



Lesiones morfologicas hemocromatosis:

1. Hepatica… cirrosis… riesgo carcinoma
2. Fibrosis pancreatica…. Diabetes mellitus
3. Pigmentacion cutanea
4. Hipogonadismo secundario
5. Pseudogota articular

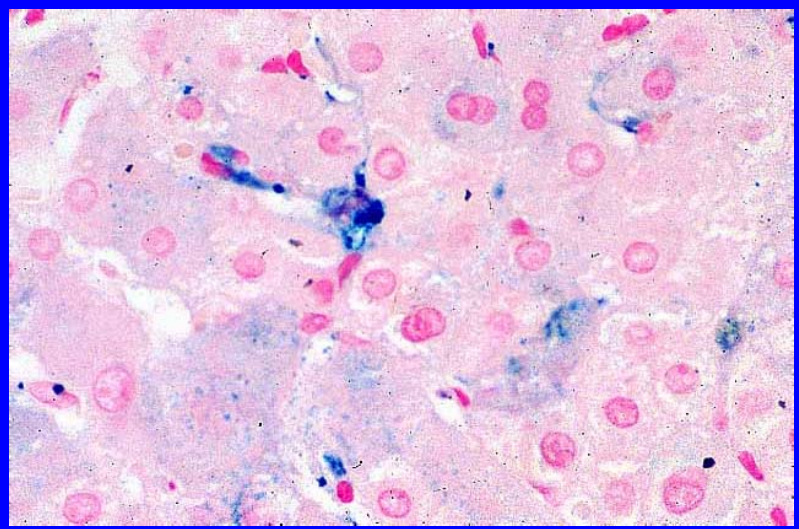


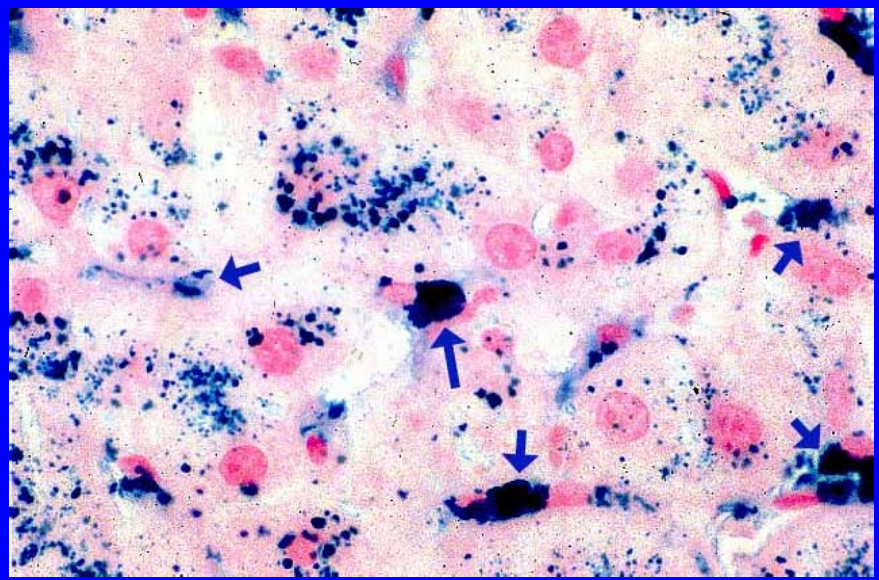
Hierro en higado:

Hepatocitos	Macrofagos	Mixto
-H.herditaria	-Transfusiones	Eritropoy.ineficaz
-Porf.Cut.Tarda	- Anemias, talas.	Mielofibrosis
-S.Porto-cava		

Macrofagos: incorporan ferritina pero no transferrina
* TfR en HEPATOCITOS y celulas en crecimiento

HEMOSIDEROSIS distinguir de hemocromatosis





Hemocromatosis avanzada: macrofagos tambien

Enf. Wilson

A.R., ATPasa de Wilson
Depositos cobre en Higado, cornea, SNC (nucleos de la base)

-hepatitis cronica….cirrosis
-Degeneracion, atrofia de nucleos de la base
-Anillo Kayser-Fleischer corneal

Enf. Poliquistica renal A.D.

1:1000

-alta penetrancia: Insuf.RnCr < 60a (40%), < 70a (75%)

-heterogeneidad genetica: 85% PKD1 @ 16p13.3

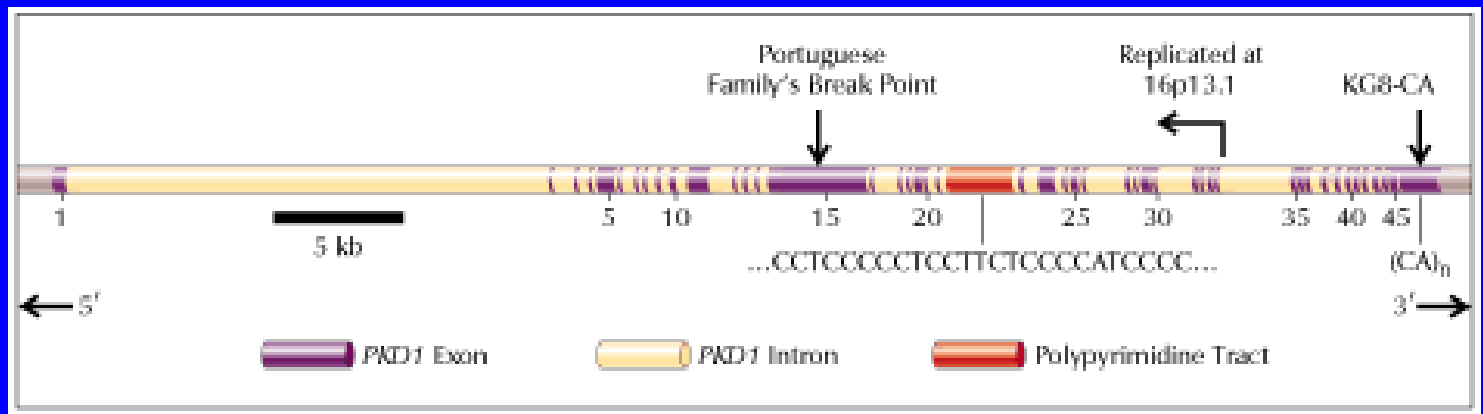
15% PKD2 @ 4q13-23 (menos severa)

-heterog.alelica: Dx. molecular directo dificil (Pseudogenes)

-quistes y malform. otros organos: enf.poliq.hepatica (40%)

prolapso mitral (20%)

aneurismas intracran. (7%)

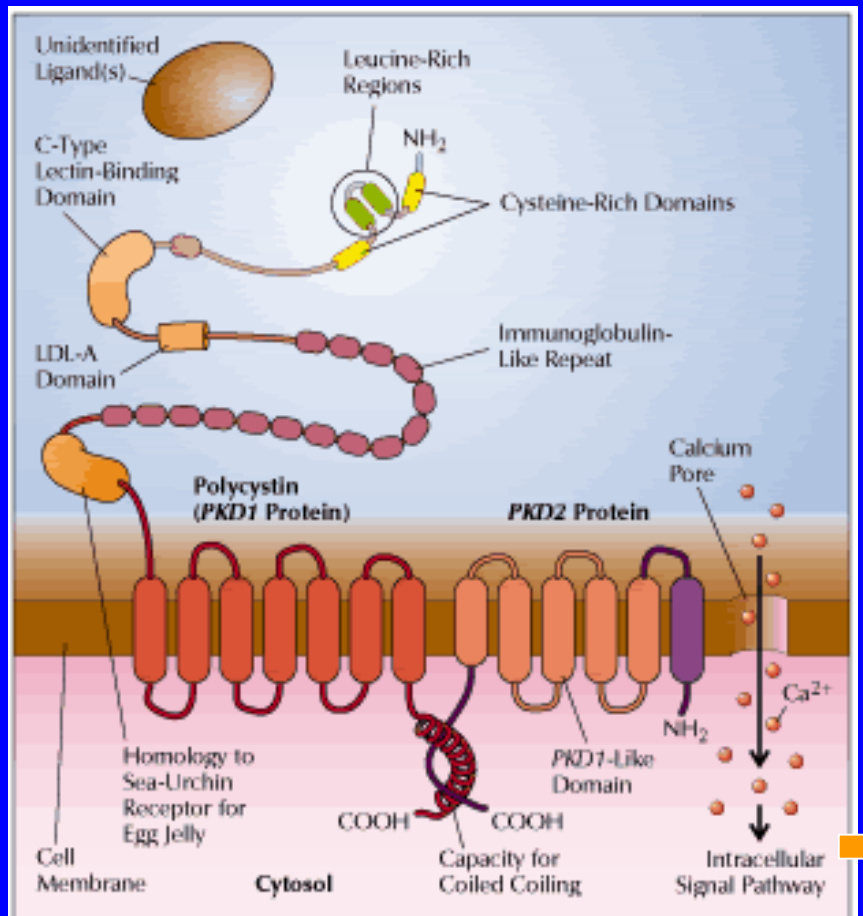


46 ex., 53 kb

14 kb mRNA

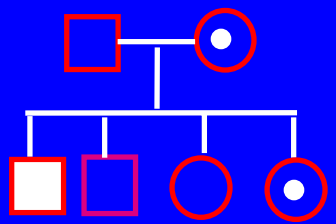
Quistes: >> proliferacion
>> apoptosis
* segunda mutacion, somatica

PKD1-PKD2:
Oncosupresor, regulacion interaccion epitelio-matriz EC



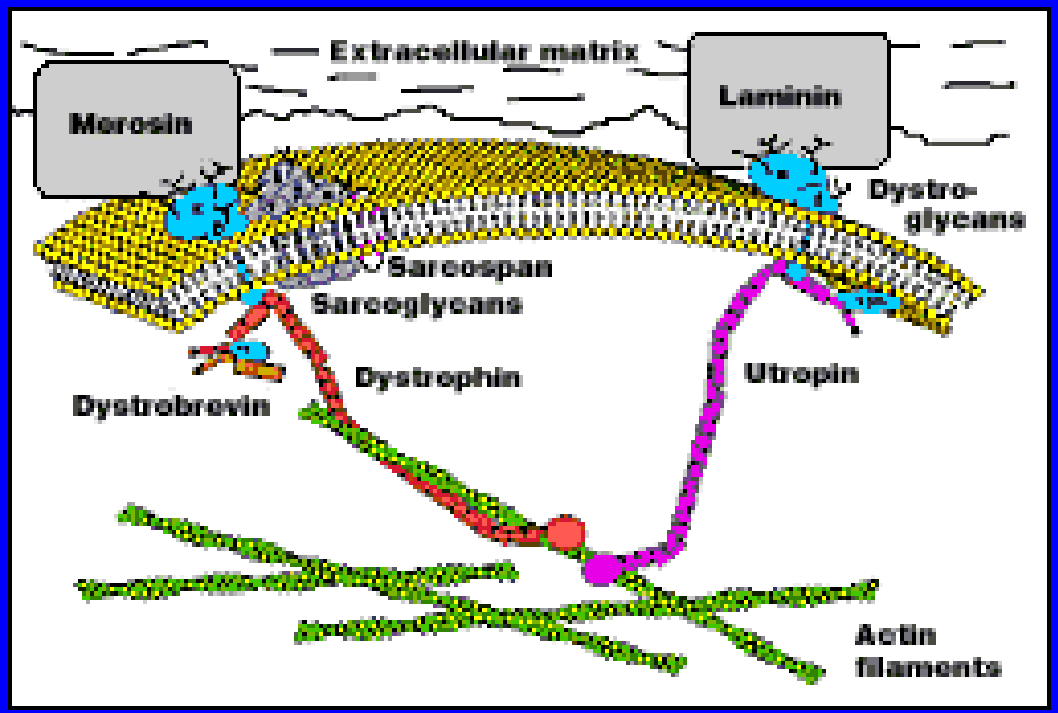
Stop G0-G1
↑
p21
↑
JAK-STAT

Enf. Duchenne / Becker



1/3 cromosomas con mutacion se pierden cada generacion

Si la incidencia se mantiene en 1:3300 varones, la frecuencia de mut.de novo debe ser 1/3 x 1/3,300 = 1/ 10,000



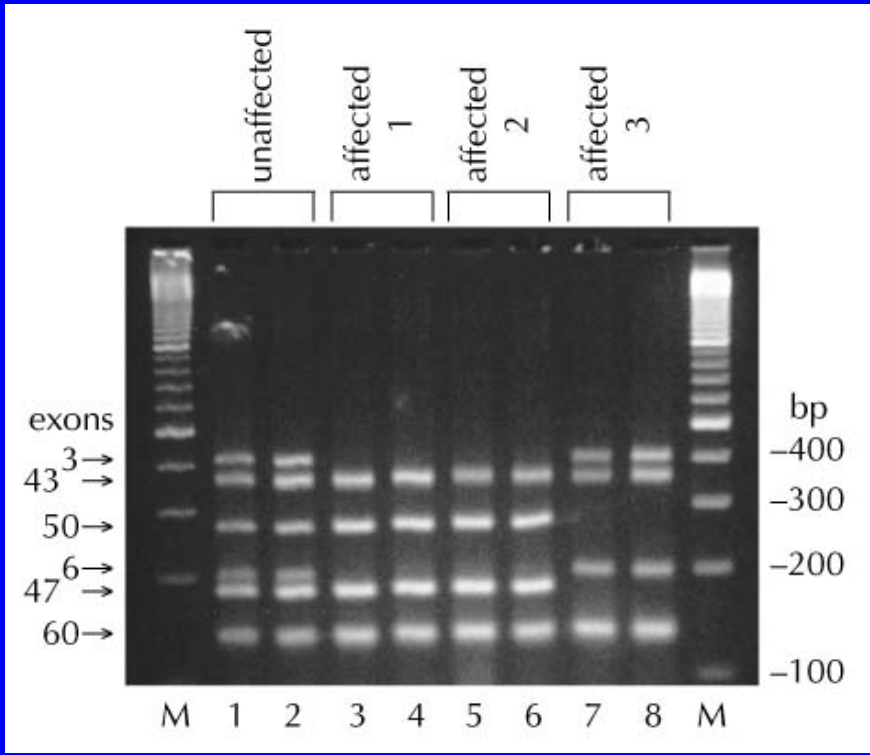
DMD:
2.3 Mbp
> 70 exones
-14 kb mRNA
-400 kDa distrofina

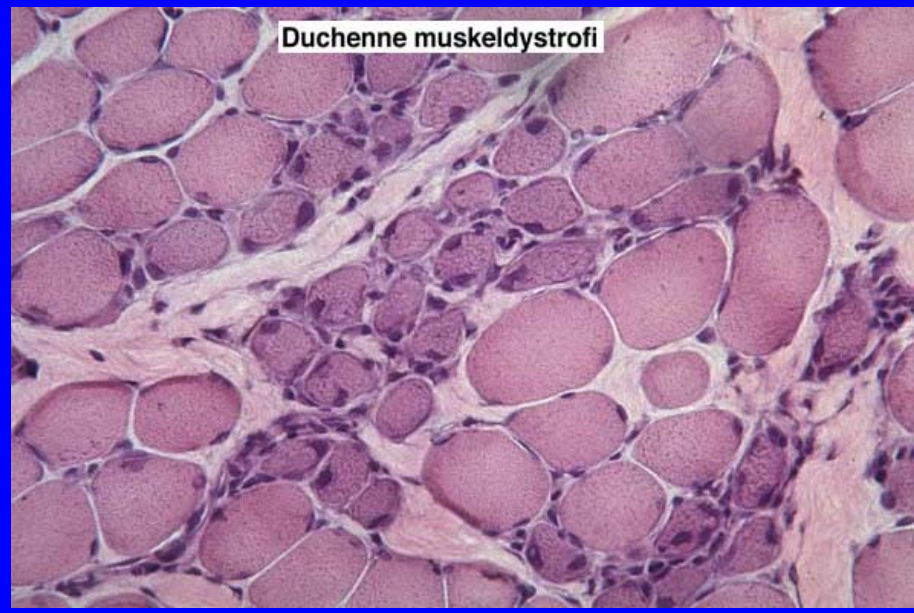
Mutacion mas frec.: deleccion de varios exones, perdida de ORF

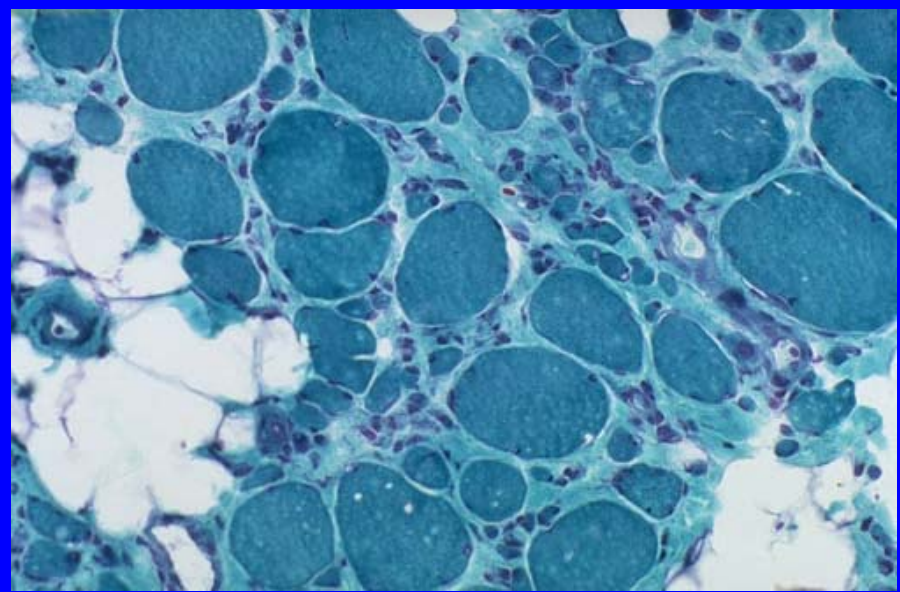
Enf. Becker: deleccion que mantiene ORF, mutacion sentido erroneo

Delecciones

PCR multiplex







Degeneracion
musculo esqueletico
Pseudohipertrofia

Enfermedades conformacionales

Sintesis Proteica

Conformacion Funcional

Conformacion No-Funcional

Respuesta a proteina malplegada

<u>Agregacion:</u>

- Anemia Falciforme
-Alfa1-Antitripsina (ZZ)
- Huntington
-Alzheimer

<u>Degradacion:</u>

- Fibrosis Quistica (DF508)
- Hipercolesterol. Fam.
- Fenilcetonuria

Enf. Huntington

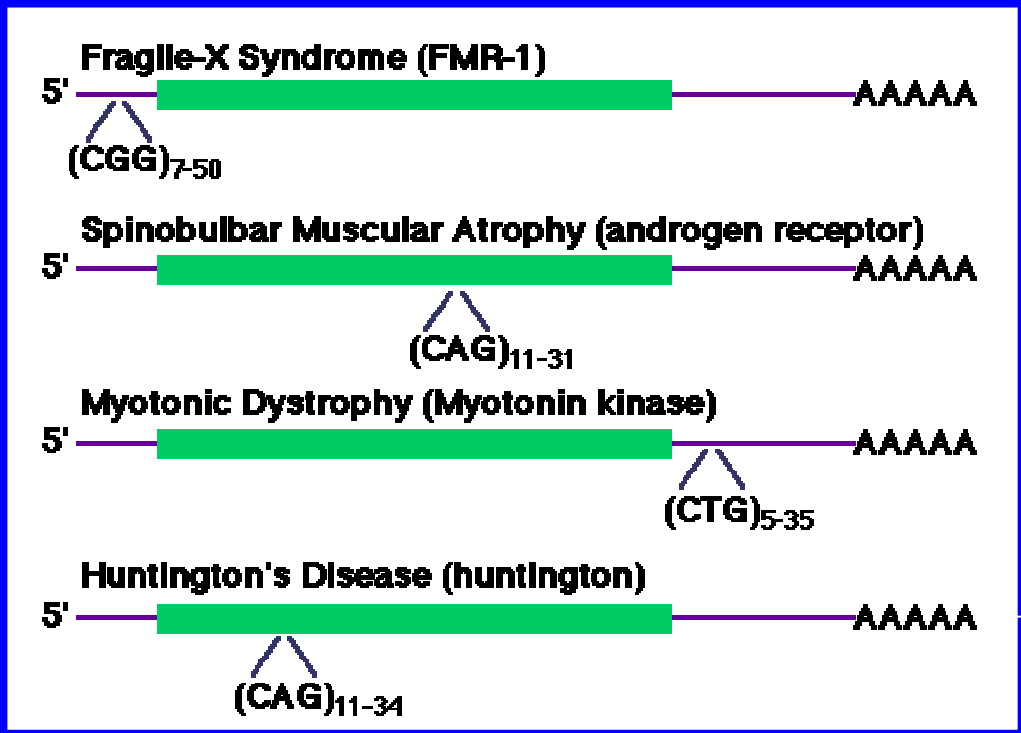
1:10-25,000

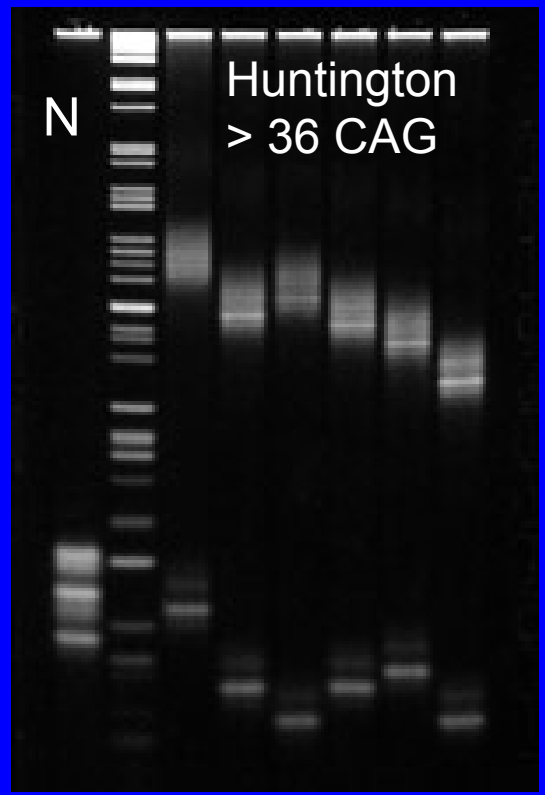
-mutacion original noroeste europeo, rara ‘de novo’

-presentacion tardia (40-50a)

-anticipacion

-formas de presentacion temprana: transmision paterna



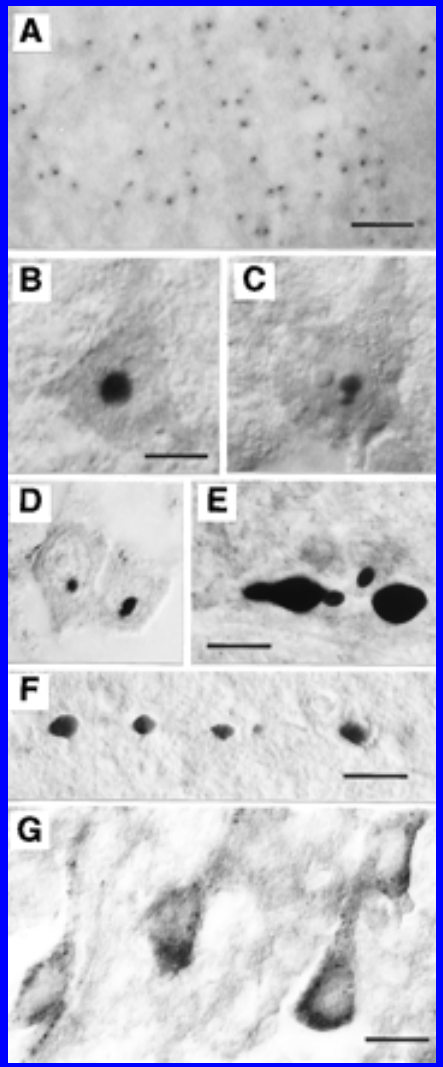


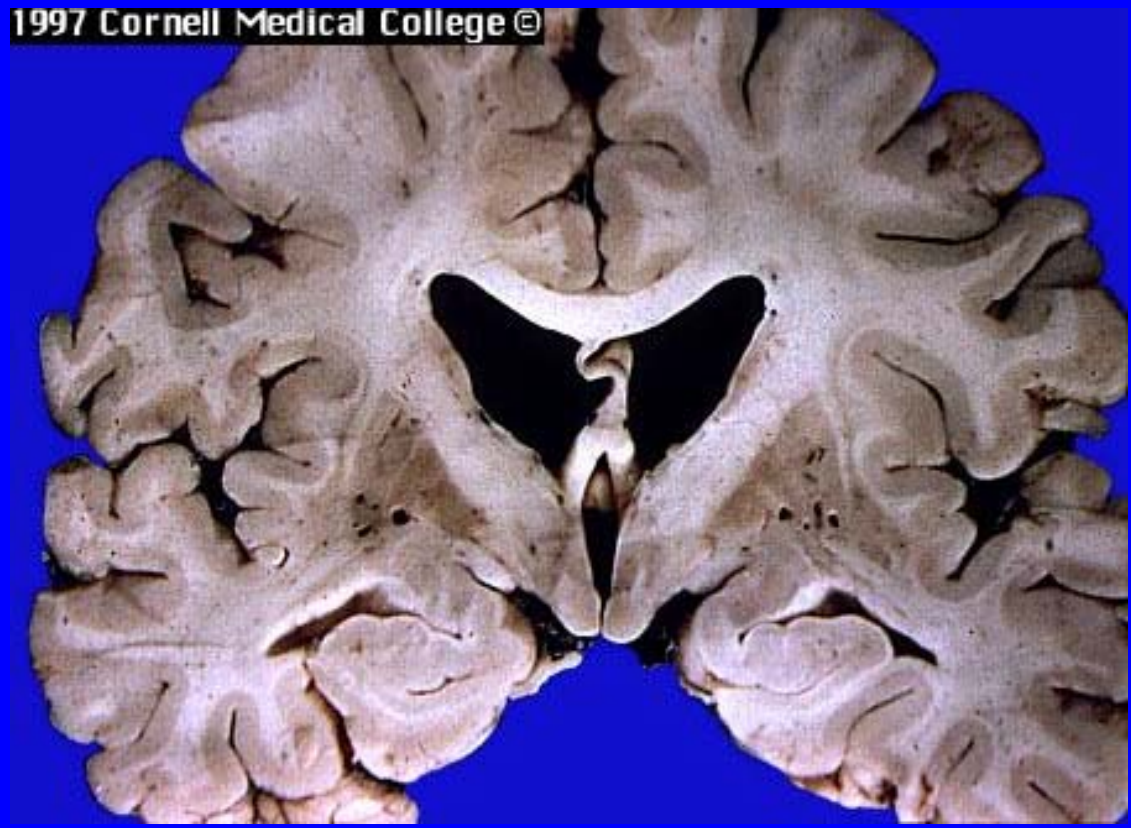
Poliglutamina…. Agregacion proteica: toxicidad

saturacion proteasoma

muerte neuronal (GABA+)

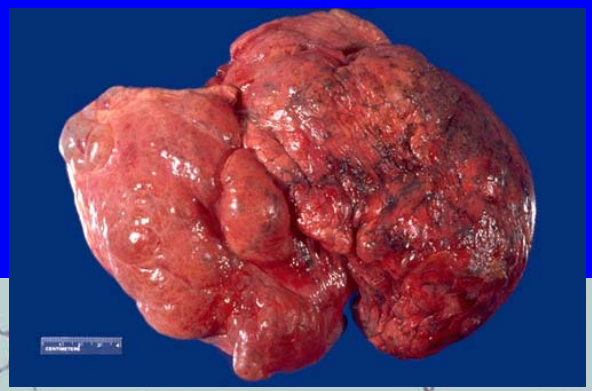
nucleos basales (caudado>putamen)

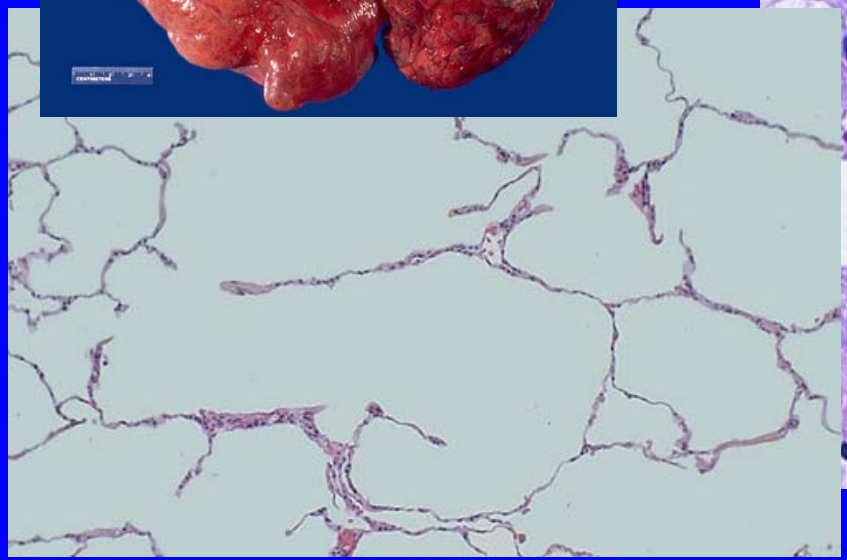


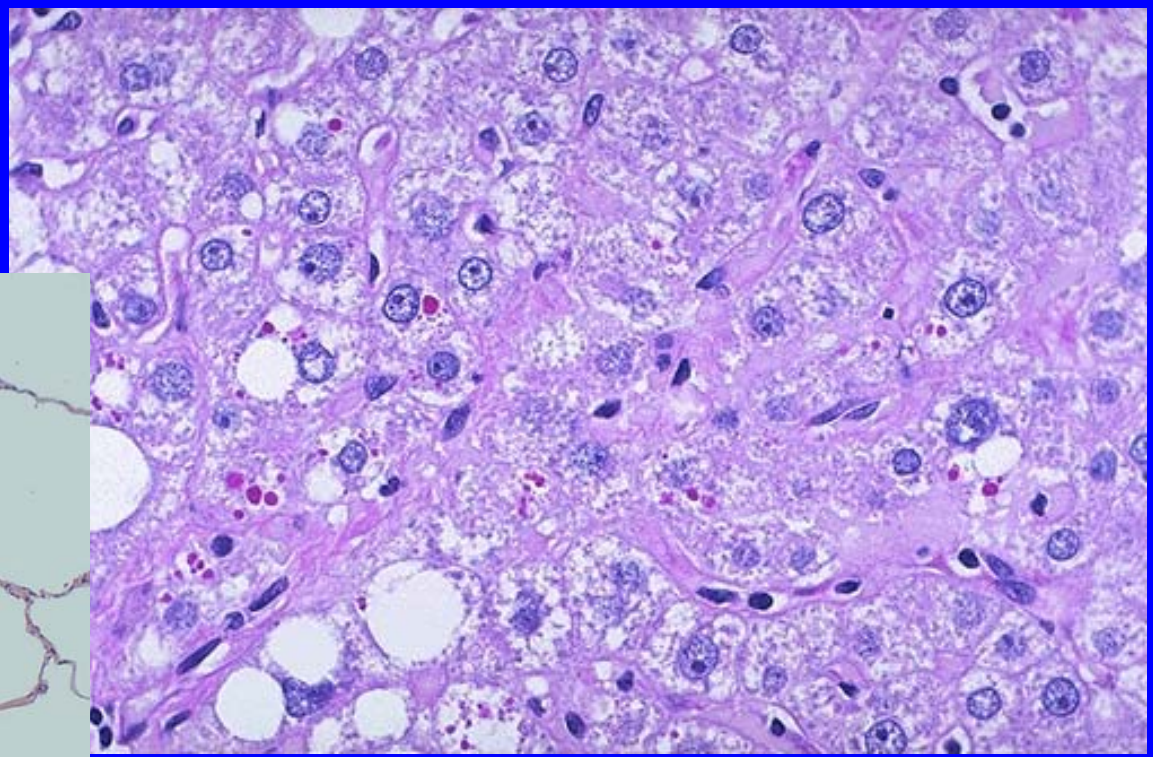


Def. alfa1-antitripsina

-gen muy polimorfico: > 75 variantes, decenas de mutaciones
-interaccion con proteasas, modelo de cambio conformacional
-insuficiente capacidad bloqueo proteasas:
    - enfisema pulmonar (tabaco): ej. enf. ECOGENETICA
-alelo Z (E342K): agregacion en R.E., cuerpos PAS+ hepatocito

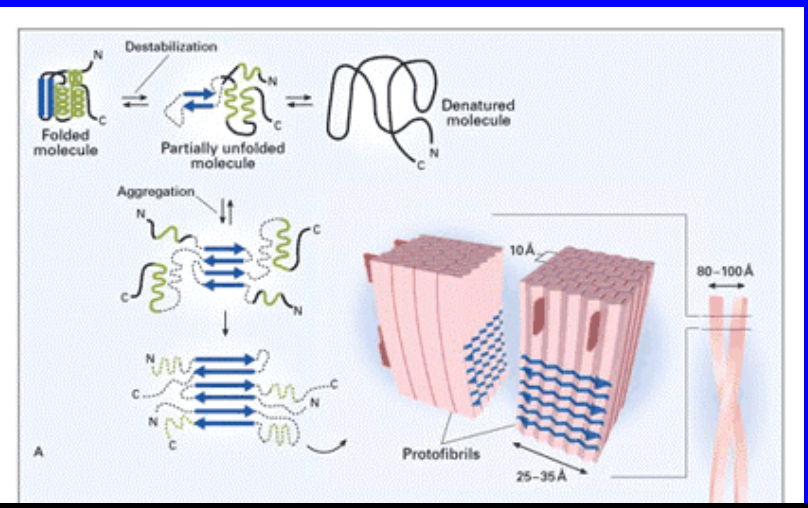






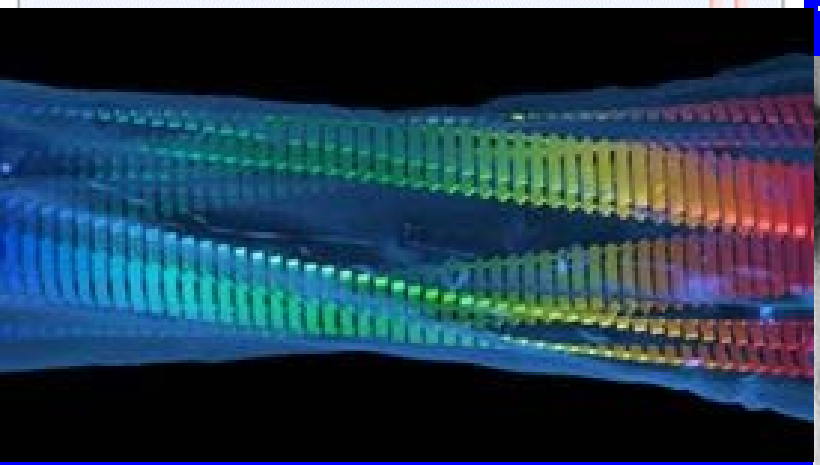
Amiloidosis

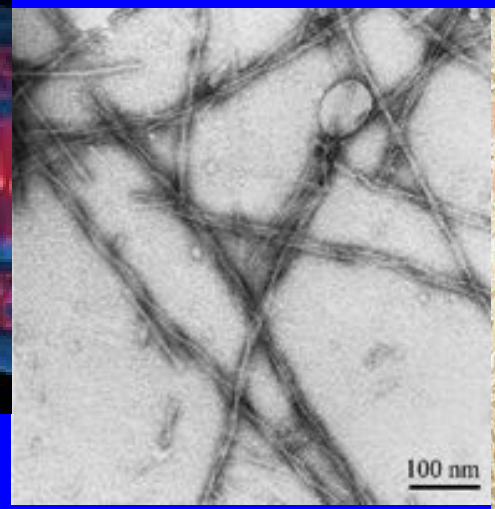
proteinas fibrilares de conformación lámina beta plegada
+ glicoproteína P sérica (PAS + ~ prot.C react.) RojoCongo+

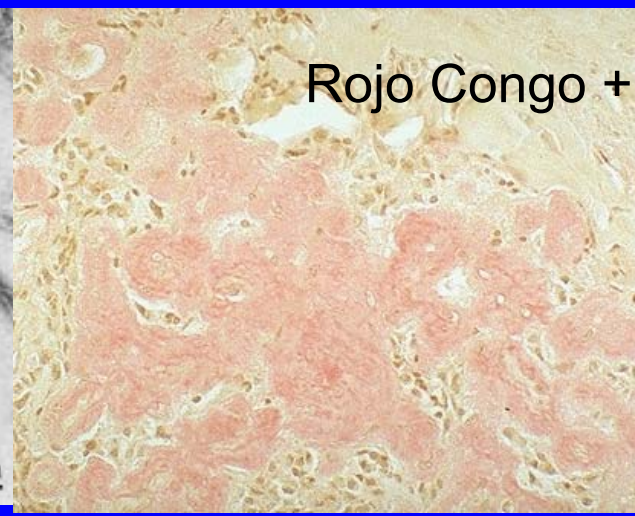


múltiples tipos:

- **AL**: de cadenas ligeras Ig (lamda > kappa)
- **AA**: de SAA (amil. asociada sérica)hepático
- **Aβ**: de APP (prot.precursora amil.) Alzheimer
- **Acal**: Calcitonina en Ca Medular tiroides
- **AIAP**: de péptido amil. del islote pancreático
- **Aβ2m**: microglobulina, hemodiálisis
- **ATTR**: transtiretina, amiloidosis familiar







Genético

(A.N.Familiar)
mutación transtiretina

↓

TTR mal plegada
Agregación
Proteolisis parcial

↓

AF

Prolif.clonal B

↓

Cad.ligeras

↓

Proteolisis parcial

↓

AL

Ambiental

Infección crónica

Inflam. crónica
(A.Reumatoide)

↓

Macrófagos: IL-1, IL-6..

↓

Hígado: SAA

↓

Proteolisis parcial

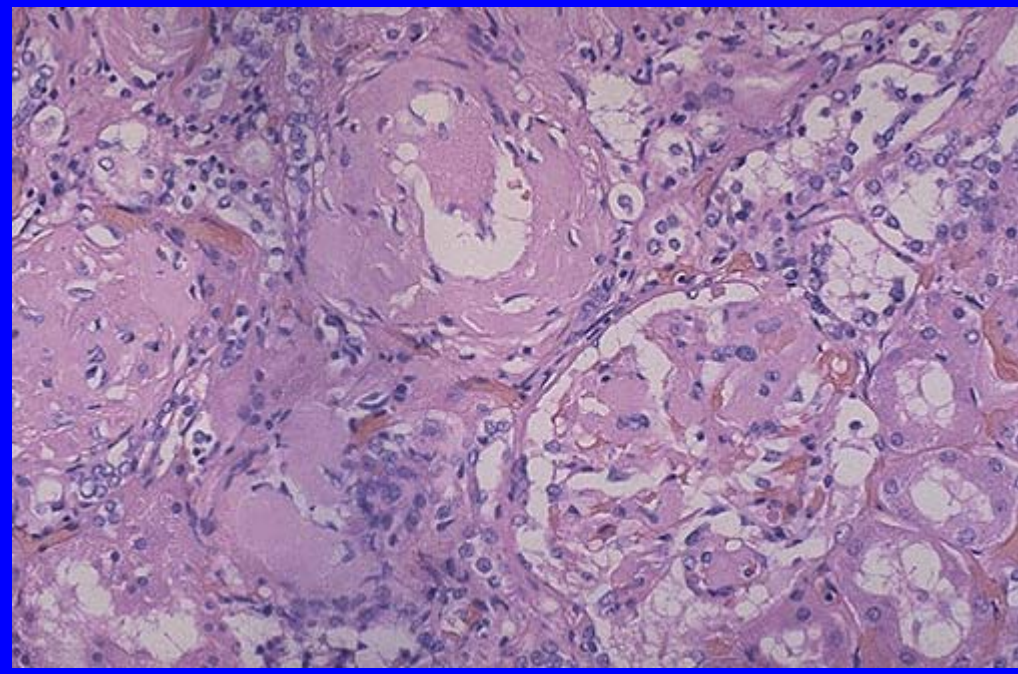
↓

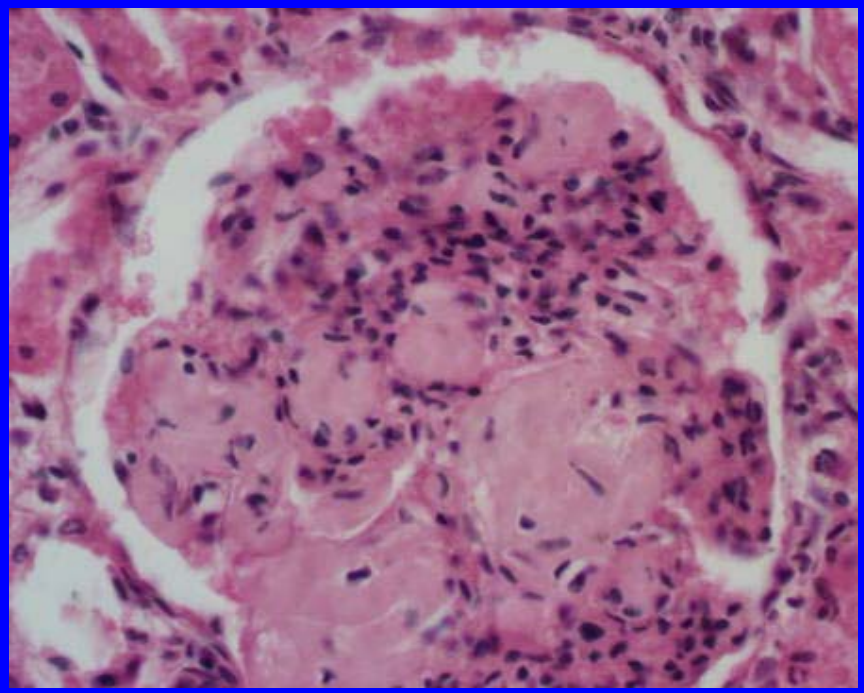
AA

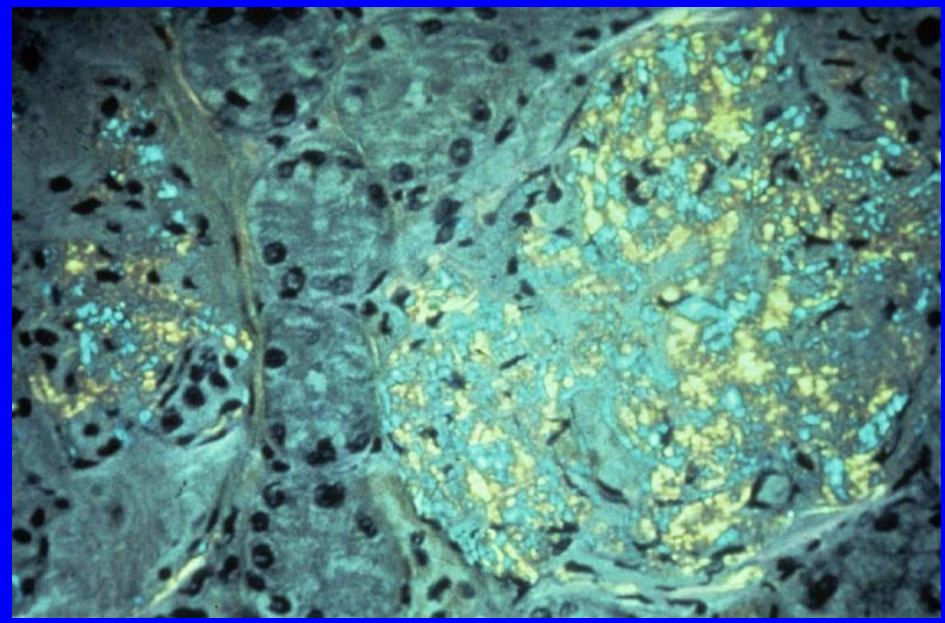
- **Primaria:** corazón, digestivo, pulmón, vasos
- **Secundaria:** hígado, bazo, riñón

Rojo Congo +
* viraje a (-) con permang.K si es secundaria (AA)

Amiloidosis renal

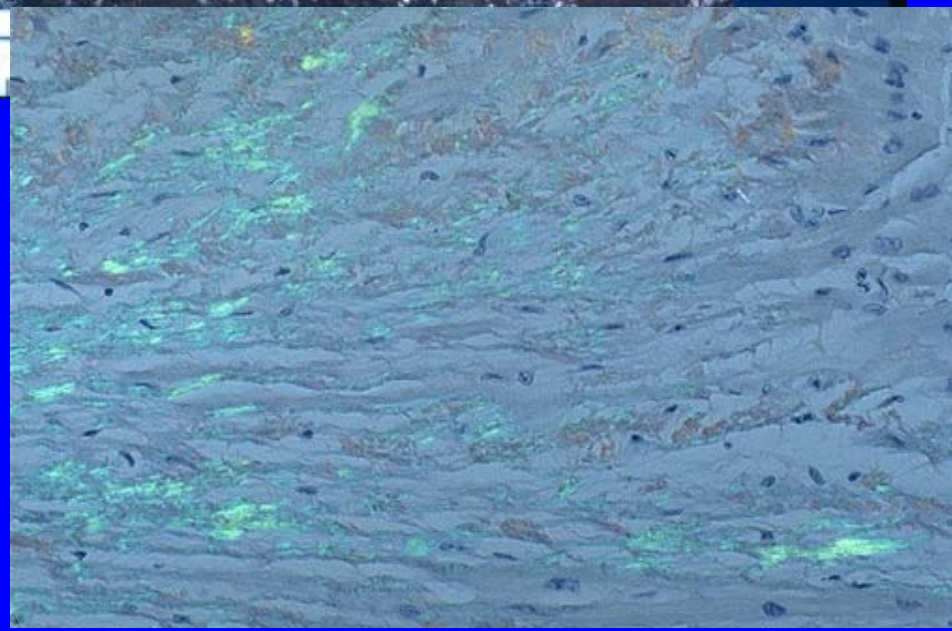




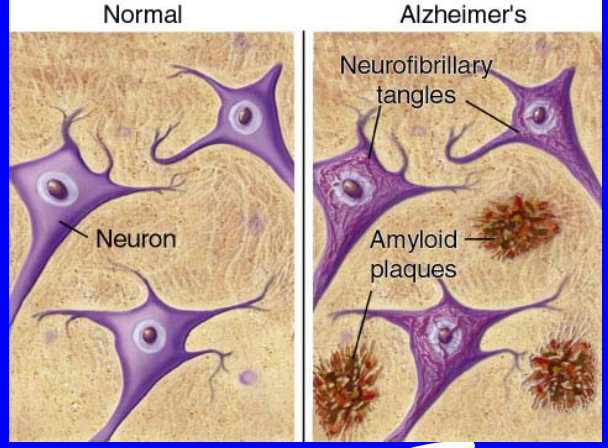


corazon

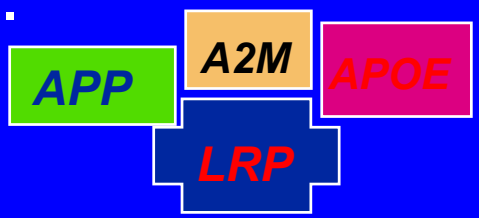
Bazo:
Sagu: foliculos
Lardaceo: sinusoides

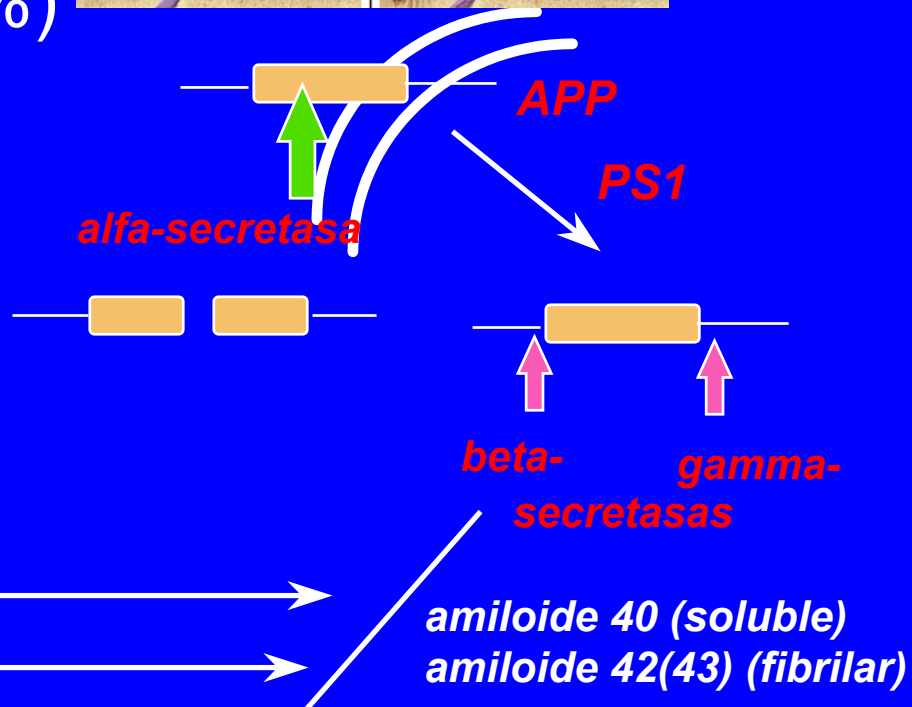


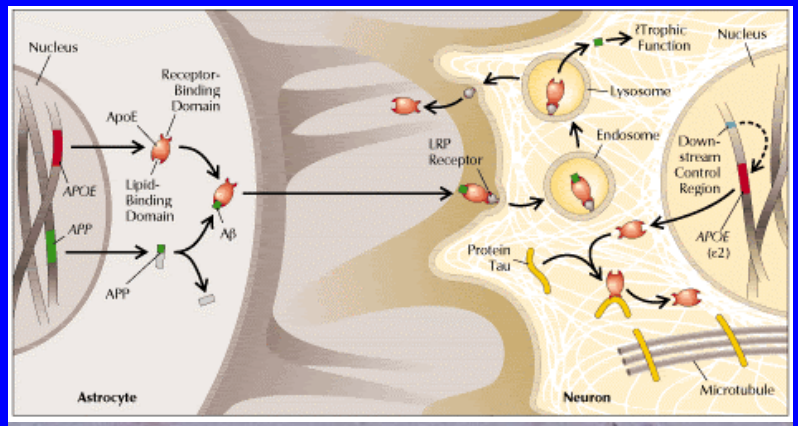
Enf. Alzheimer

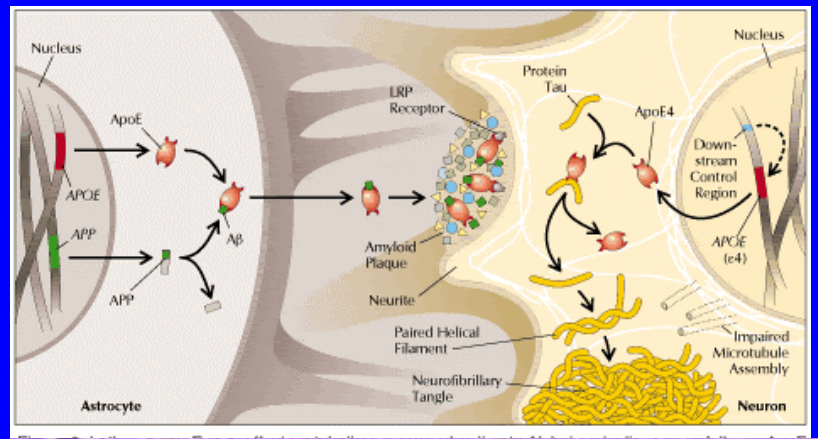


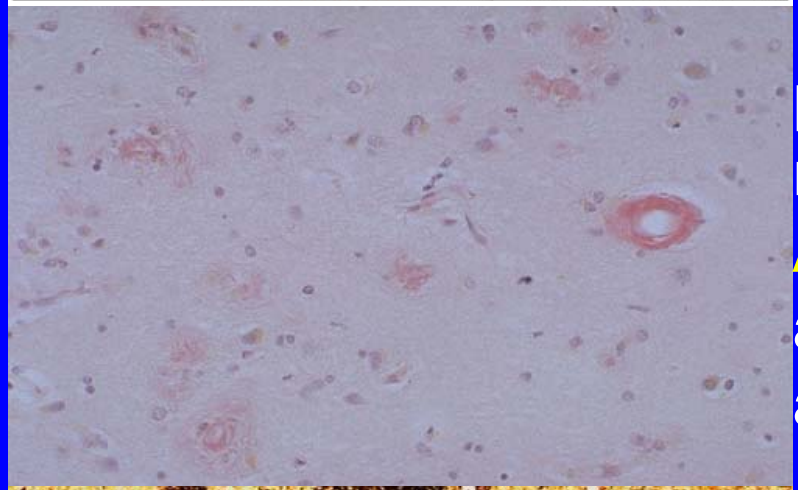
- Familiar (A.D.) (<10%)
  - APP
  - PS1
  - PS2
- Esporádica (>90%)
  - APOE
  - A2M
  - LRP
  - ....



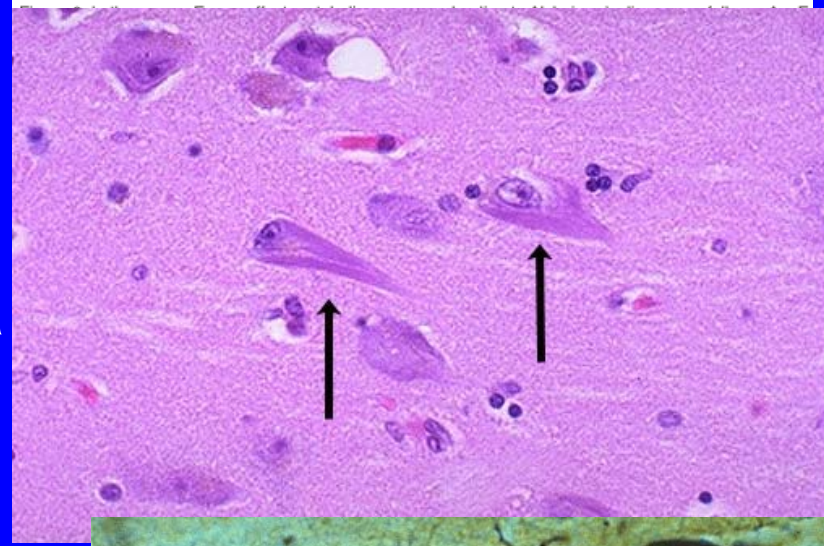


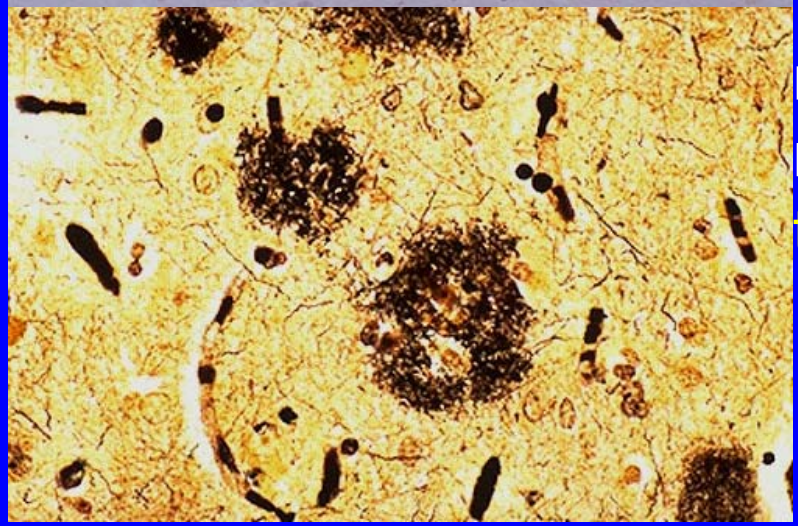




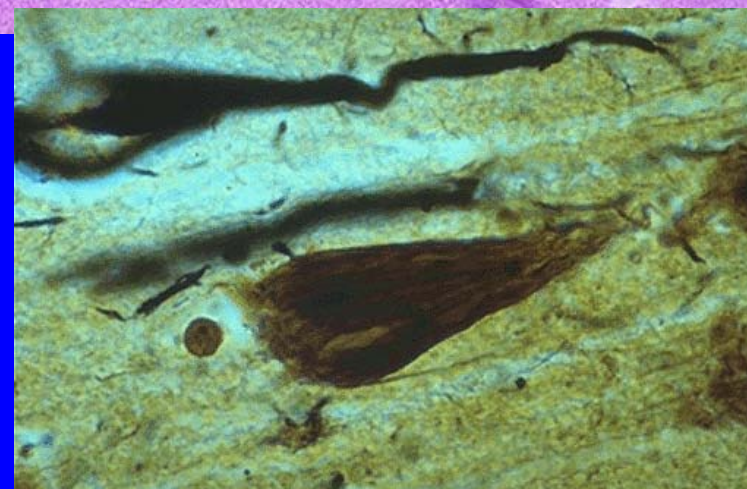


placa neuritica AB4 angiopatia amiloide

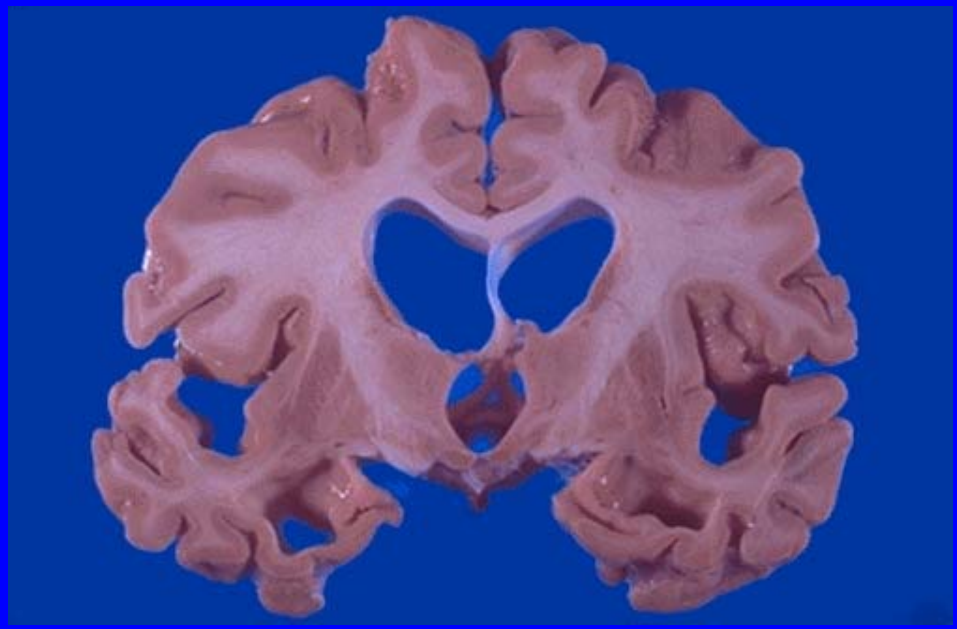




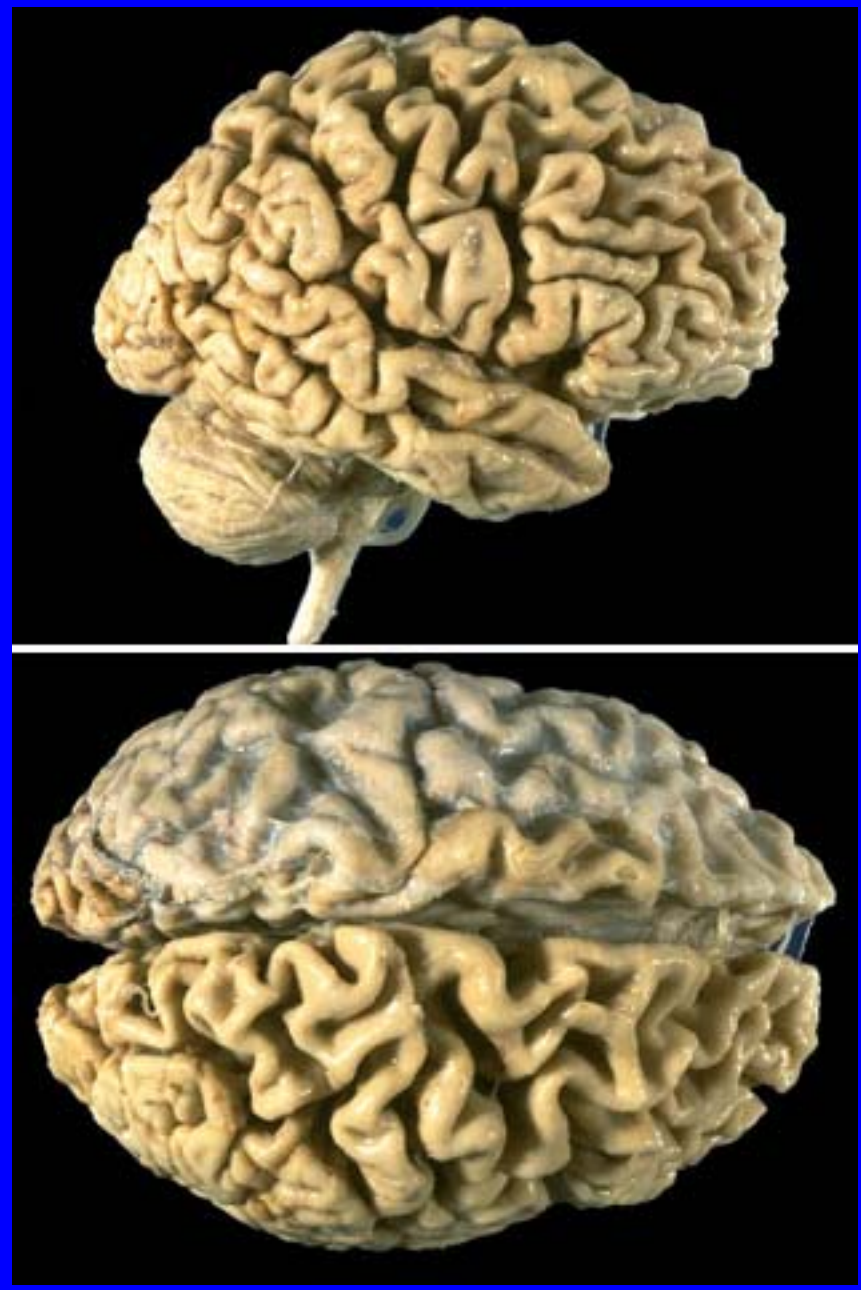
marana neurofibrilar tau



Alzheimer:



Atrofia cortical

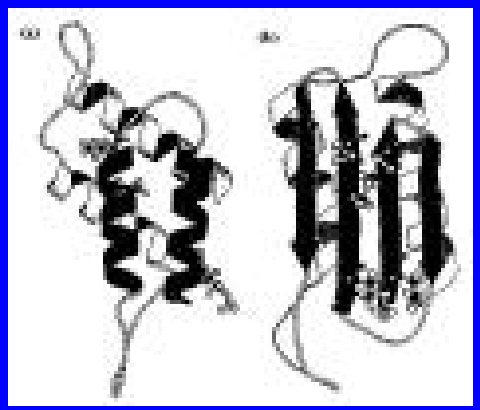


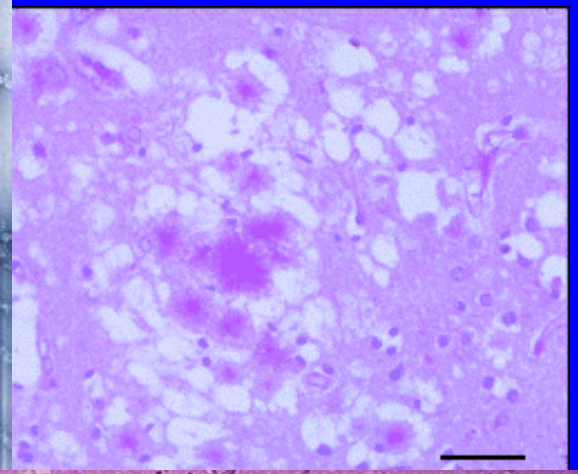
Priones

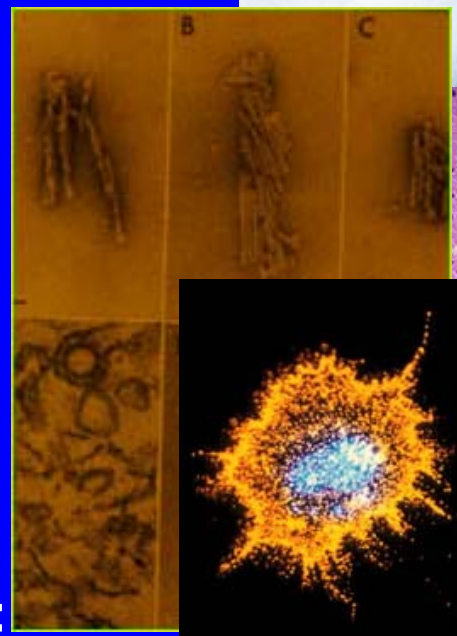
Formas anormales de PrP, transmisibles

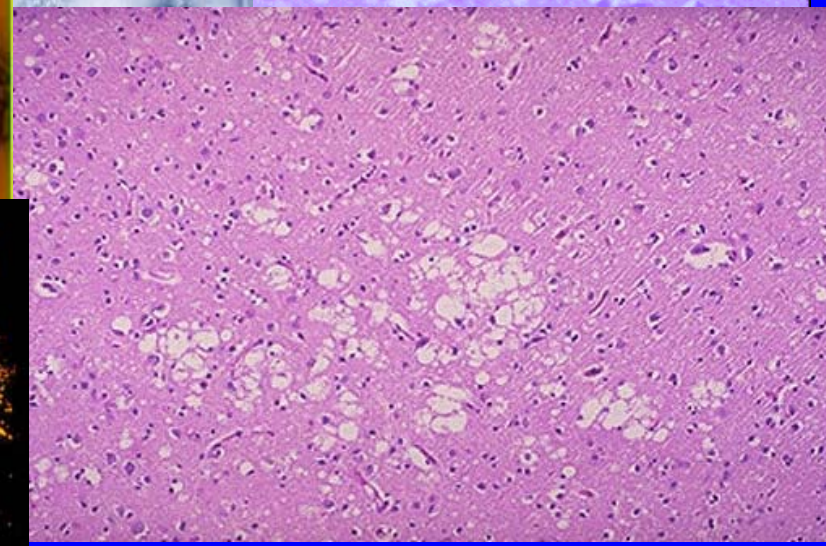
transformacion alfa-helice (PrP^{c})

>> lamina beta (PrP^{sc}), proteasa-R









CREUTZFELDT-JAKOB:

-espontanea (muy lenta)

-familiar: mutaciones en *PRNP* (ej. D178N)

- con VV o VM @ 129 > CJD fam.
- con MM o VM @ 129 > insomnio letal fam.

-variante (vCJD), transmision de BSEncefalopat.

Diabetes Mellitus

Deficit Insulina: absoluto / relativo → Mala utilizacion carbohidratos → Glicosilacion no enzimatica → Lesiones Vasculares

DM tipo 1

-juvenil

-deficit absoluto Insulina

-30-50% concordancia univitelinos

-autoinmune, HLA DR3 y 4

DM tipo 2

-adulto

-deficit relativo Insulina

-80% concordancia univitelinos

-resitencia periferica a insulina

f. geneticos:

-HLA-DR3, DR4

-HLA-DQb Asp57(-)

-TCR

-Insulina

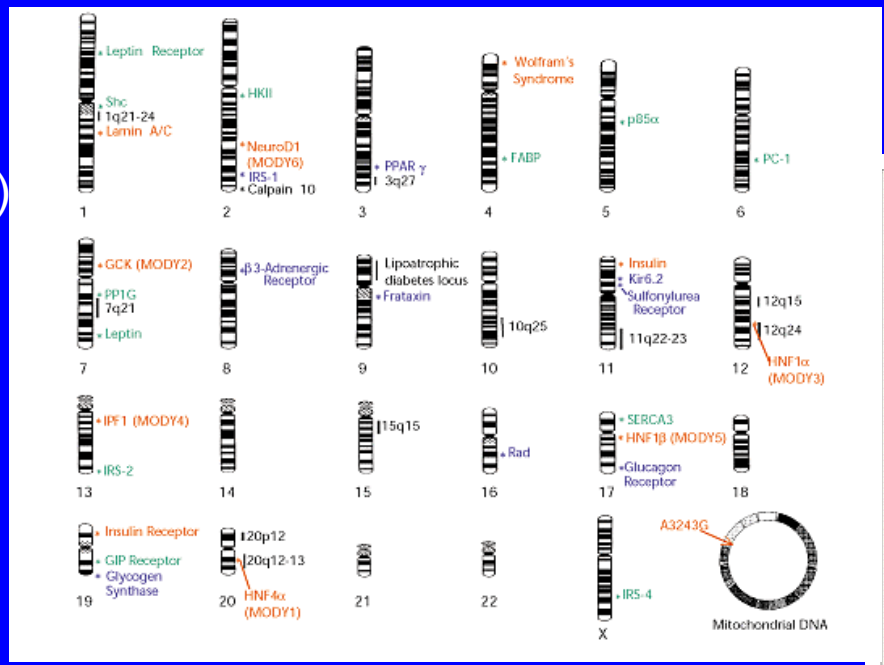
-...

f. ambientales:

-virus (Coxsackie

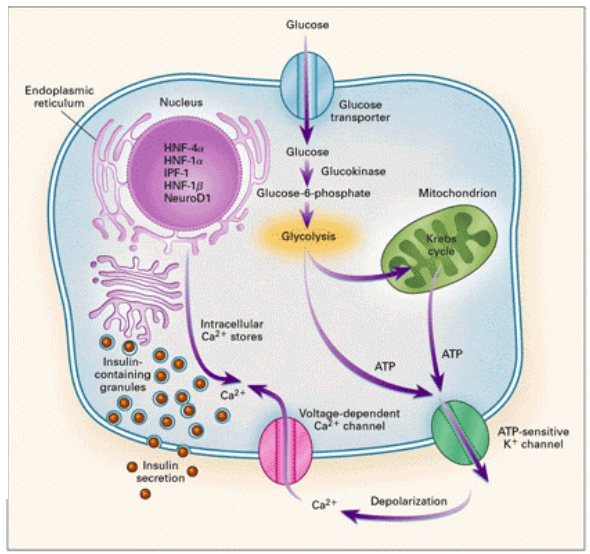
-obesidad

-embarazo

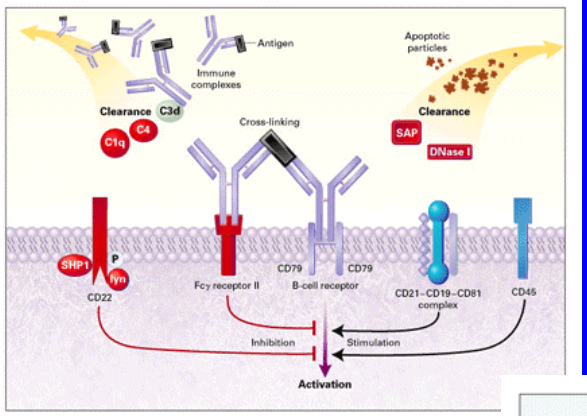


MODY: (A.D.)

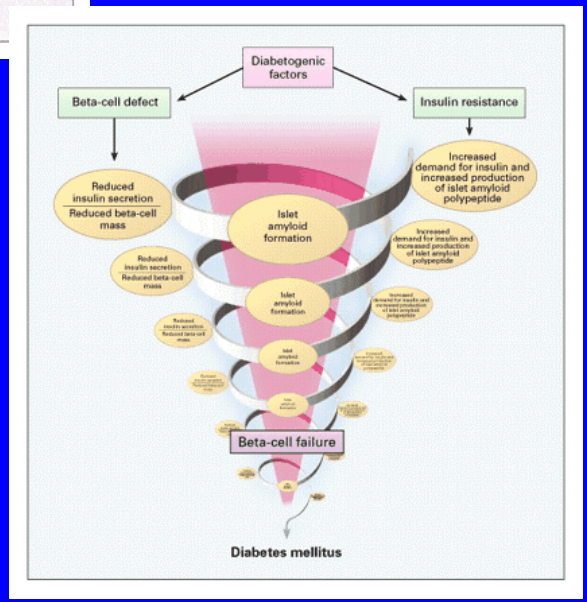
HNFs, GK, mit.DNA…



reac.autoinmune (DM t1)
-anti-GAD (CD4+ Th1)
- anticuerpos: GAD, IA-2, In…

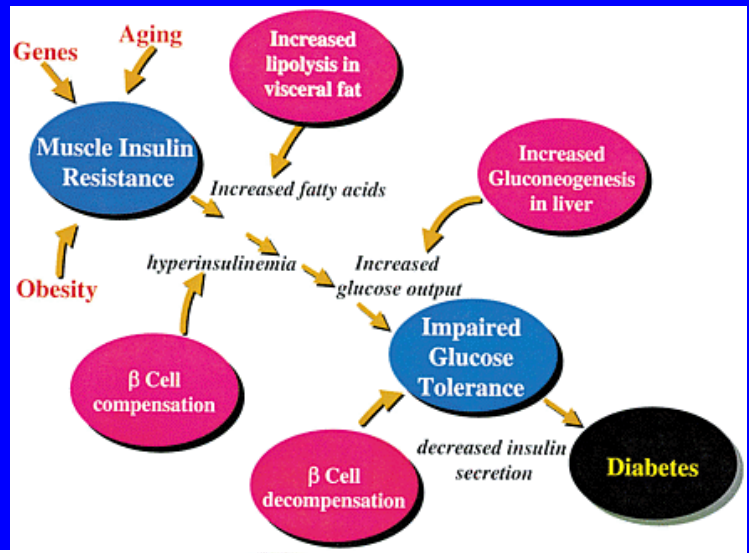


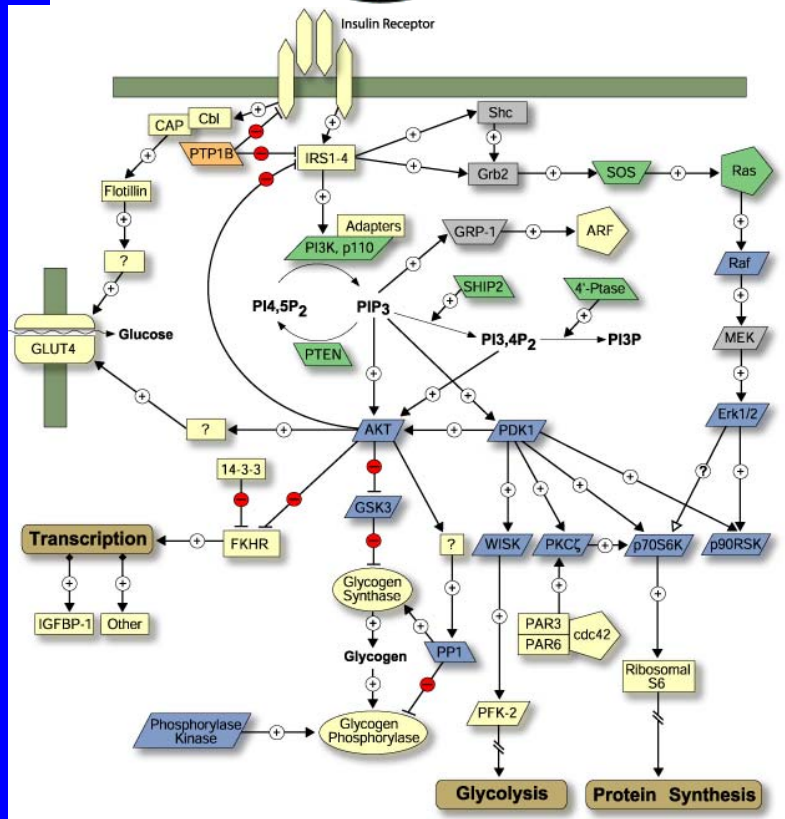
Insulitis
destruccion cel.beta



Def. absoluto / relativo Insulina
= hiperglucemia

resistencia periferica (DM t2)





hiperglucemia

Glicosilacion no enzimatica

puentes (cross-linking):

- Membr.Basal capilar
- Receptores: proliferacion
- HbA1c

Lesion vascular microangiopatia engrosamiento MB

Entrada Ins-independiente

- Nervios
- Cristalino

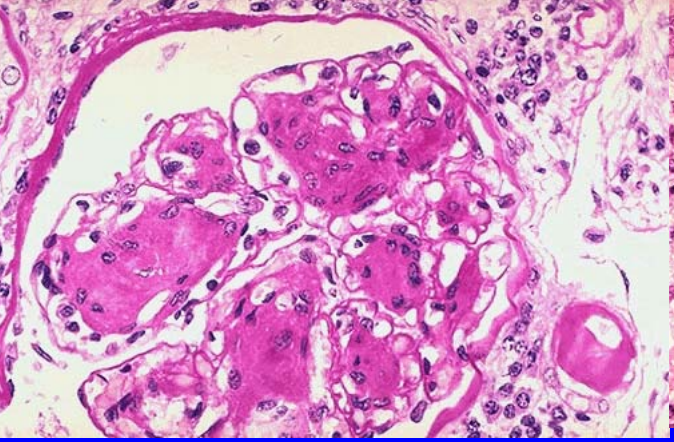
Lesion osmotica (sorbitol)

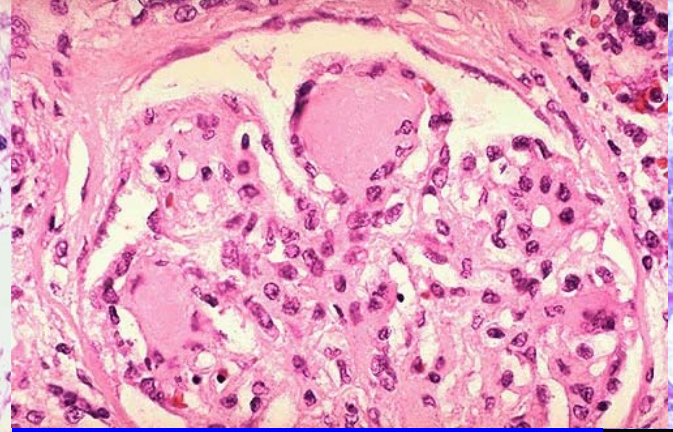
Potenciacion otras vias energeticas

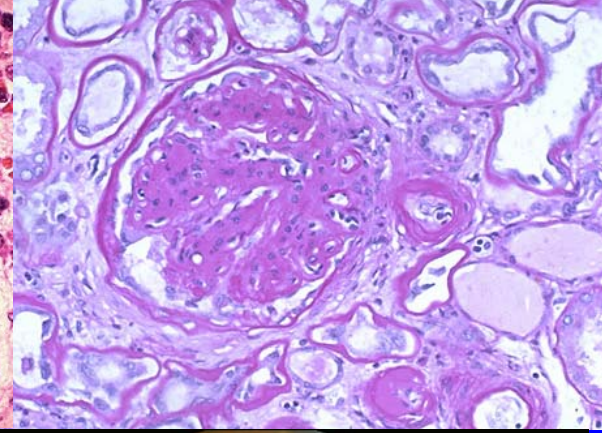
- Lipolisis
- Glucogenolisis

C.Cetonicos

1. Arteriosclerosis: precoz, severa
2. Nefropatia: glomerulosclerosis, arteriolosclerosis, pielonefritis, tubulopatia
3. Pat.Ocular: retinopatia, cataratas, glaucoma
4. Neuropatia periferica (motora, sensitiva)
5. Pat.Pancreas: insulitis, amiloide (amilina), alt.granulos ins.

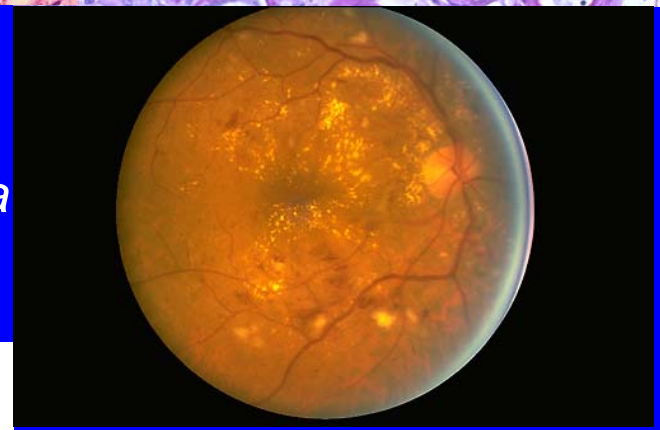


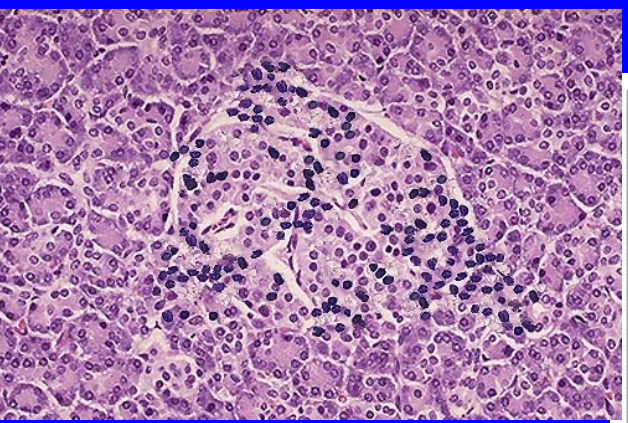




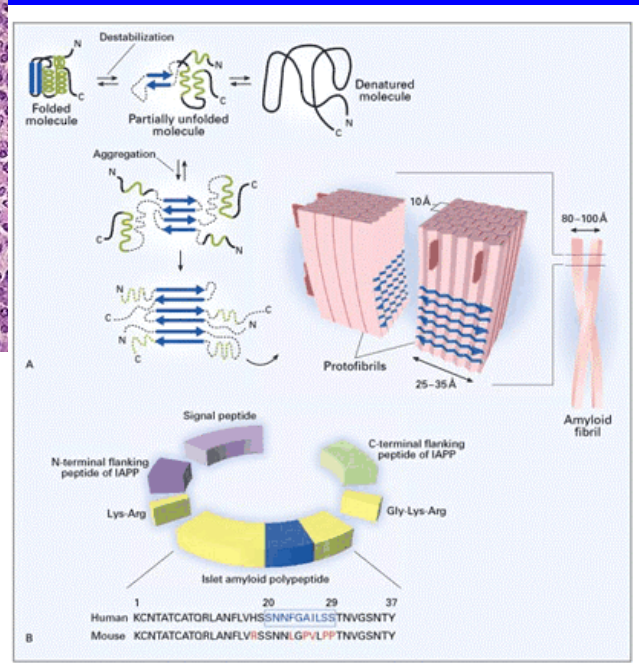
Glomerulosclerosis: - nodular (K-W) – difusa
Arteriolosclerosis: A.A y A.Eferente

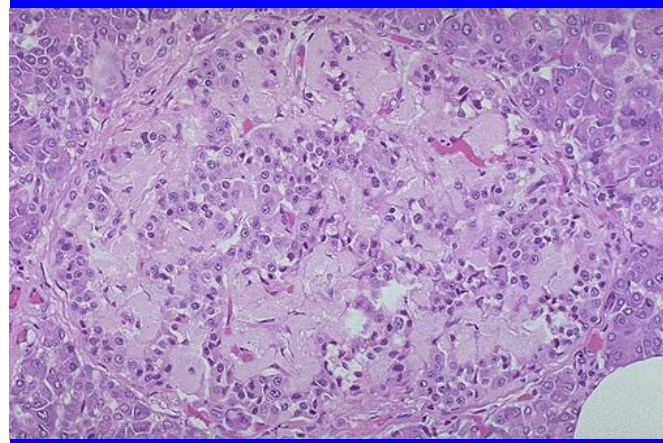
Retinopatia





Insulitis





Amiloide (amilina)

Gota

Inflamacion articular aguda o cronica (tofo) por hiperuricemia

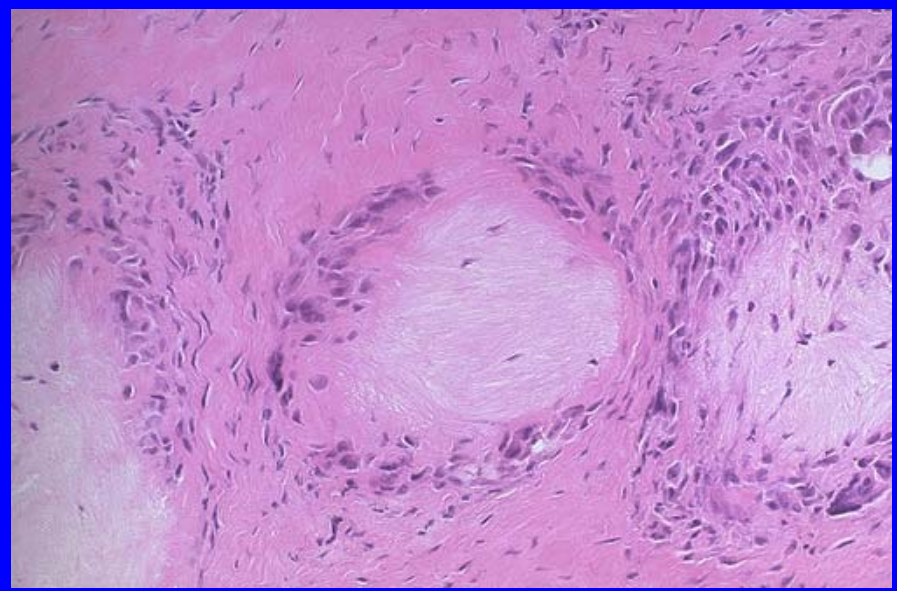
-fact.geneticos: multifactorial
excepto Def. HPRT, lig-X, S.Lesh-Nyhan

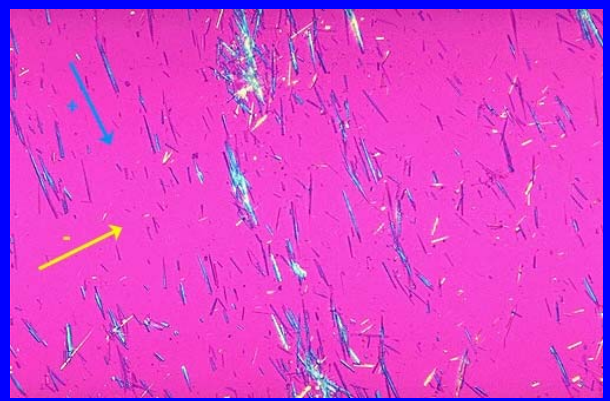
-fact.ambientales: dieta, alcohol, hiperuricemiantes, antineopl.

hiperuricemia → precipitantes

-temperatura
-comp. sinovial

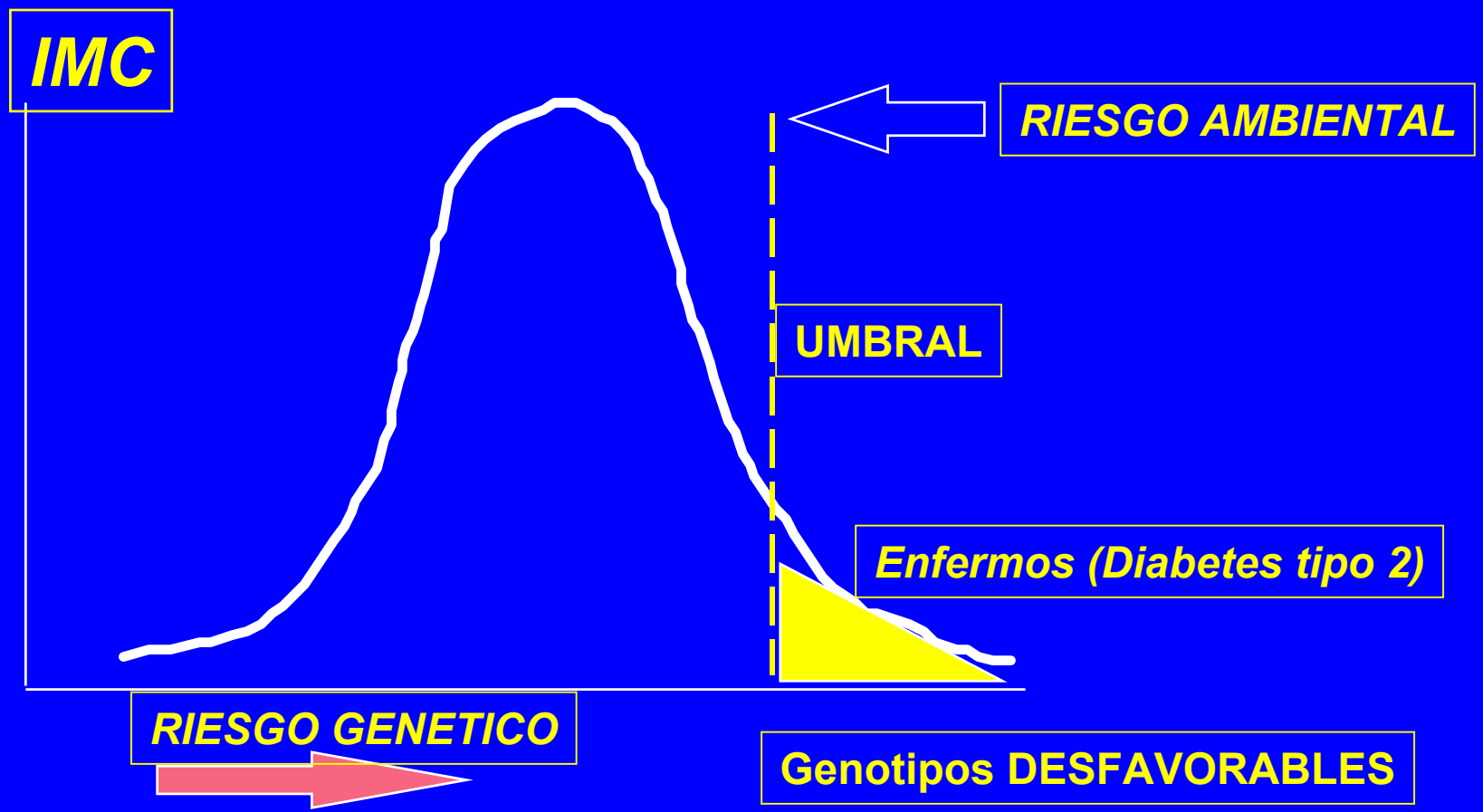
1. Artritis: aguda, cronica (tofo)
2. Pat.Renal:
   - nefropatia urato
   - insuficiencia renal aguda
   - calculos



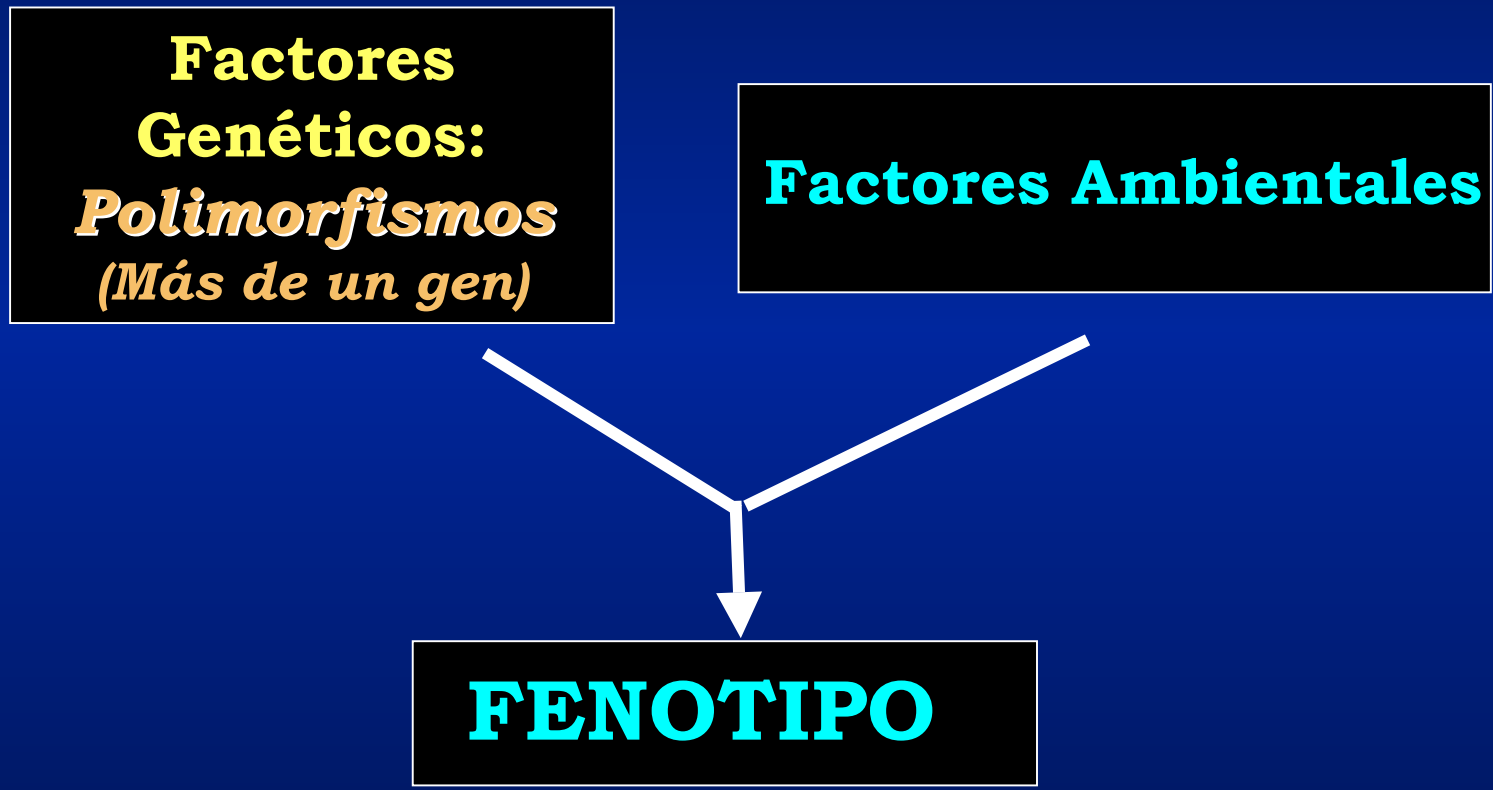


Enf. Multifactoriales

características de variación continua



ENFERMEDADES COMPLEJAS Ó MULTIFACTORIALES



Medicina genómica

- Predicción del Riesgo de Enfermedades Comunes: Prevención
- Mejor conocimiento del pronóstico: Individualización del Tratamiento
- Farmacogenómica:
  - ✓ Selección del Fármaco
  - ✓ Dosis e intervalos
- Mecanismos de enfermedades comunes