Hospital Universitario
Ramón y Cajal

Comunidad de Madrid

Enfermedades Musculares
en la
Infancia y Adolescencia (X)

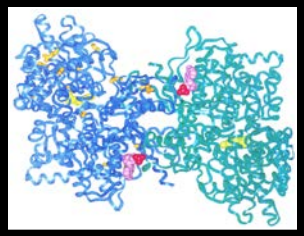
VIERNES 22 de Marzo

MESA REDONDA: Miopatías en el niño y el adolescente (IV)

ciberer
CENTRO DE INVESTIGACIÓN BIOMÉDICA EN RED DE ENFERMEDADES RARAS
U. 723

Hospital Universitario 12 de Octubre
SaludMadrid

i+12
Instituto de Investigación
Hospital 12 de Octubre



Glucogenosis tipo V (Enfermedad de McArdle): Aspectos Genéticos y Fisiopatológicos

MIGUEL ANGEL MARTIN CASANUEVA
LABORATORIO DE ENFERMEDADES MITOCONDRIALES Y NEUROMETABÓLICAS
S. BIOQUIMICA CLÍNICA. i+12.
HOSPITAL UNIVERSITARIO 12 DE OCTUBRE. MADRID

Spanish Consortium for McArdle's disease

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de Madrid

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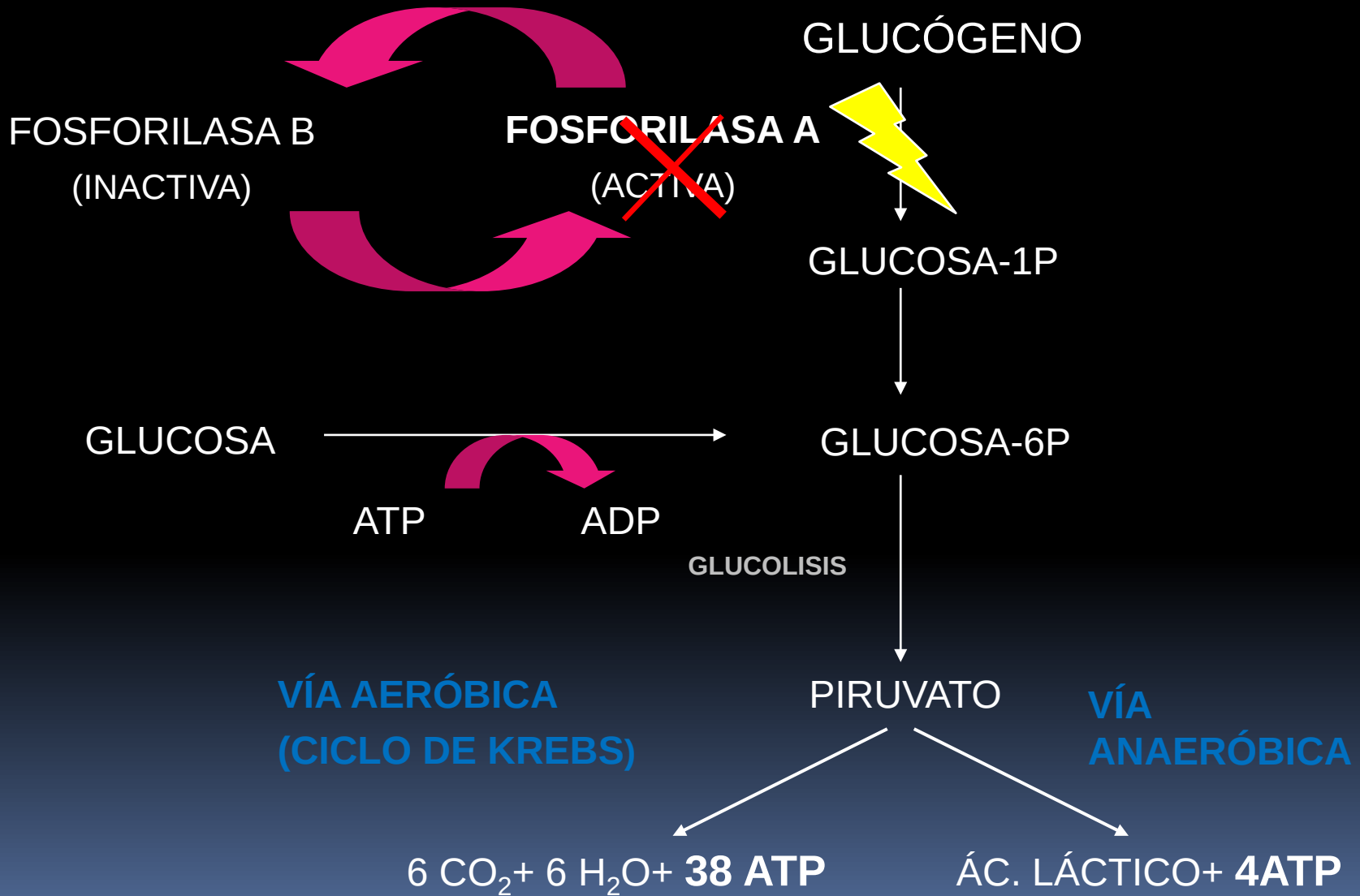
DE ENFERMEDADES RARAS

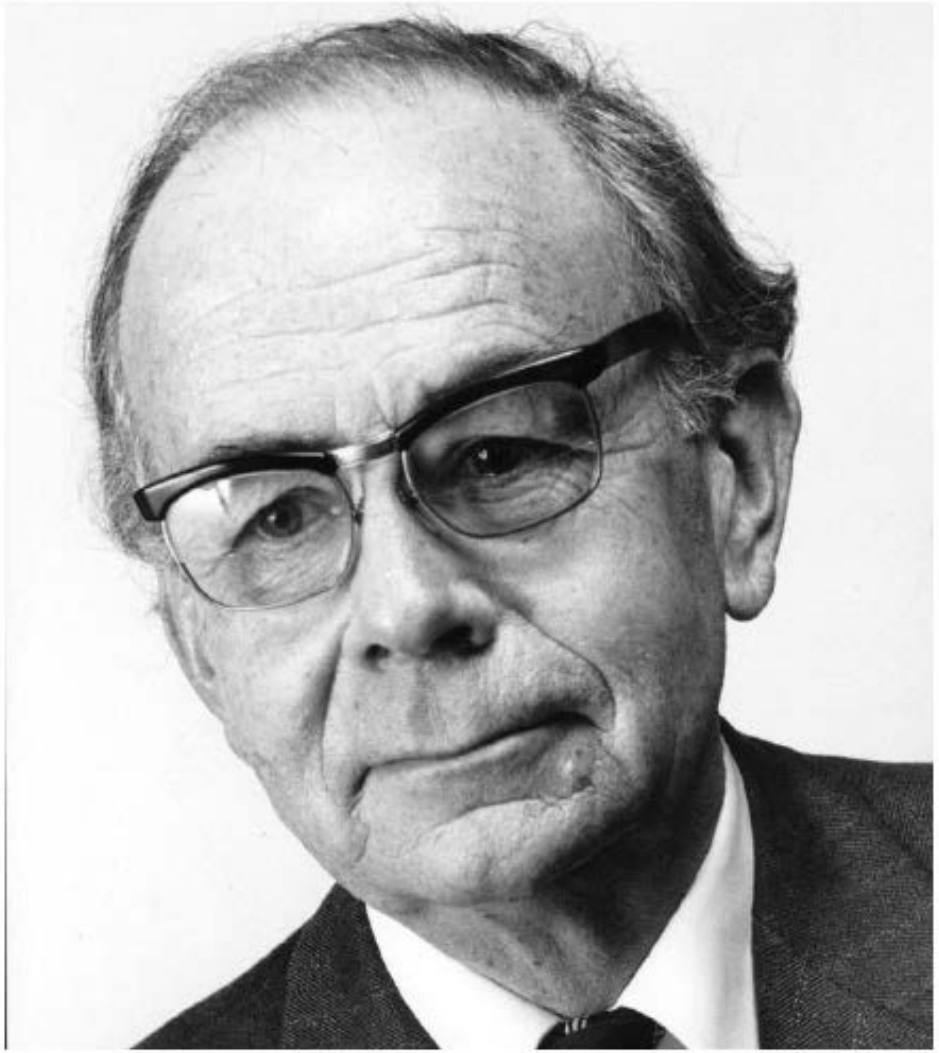
Enfermedad de McArdle
Función Miofosforilasa



Dr. Brian Mc Ardle

English Physician
and neuroscientist

1951



ENFERMEDAD DE McARDLE

DÉFICIT DE MIOFOSFORILASA (glucogenosis tipo V)

- Prevalencia estimada: 1 / 100.000 Texas. ; España ≈ 1/ 170.000
- Herencia autosómica recesiva

- Mialgias, fatiga prematura y calambres por dos tipos de ejercicio:
  - ejercicio breve de gran intensidad (isométrico)
  - ejercicio aeróbico de intensidad moderada/alta y mayor duración

- Fenómeno Segunda entrada (Second Wind): readaptación fisiológica y bioquímica muscular para utilización primordialmente de glucosa sanguínea, y ácidos grasos.

- Mioglobinuria (50%)

- Inicio: infancia
- Diagnóstico: 2ª-3ª decada, >50 a (debilidad fija)

Management

- No specific treatment exists.
- Avoid strenuous (anaerobic or sustained) exercise, including lifting or pushing.
- Ingestion of sucrose improves exercise tolerance and may protect against exercise-induced rhabdomyolysis
- Programmed and controlled aerobic exercise.

J Neurol Neurosurg Psychiatry (2012).

RESEARCH PAPER

Genotypic and phenotypic features of McArdle disease: insights from the Spanish national registry

Alejandro Lucia,[1] Jonatan R Ruiz,[2,3] Alfredo Santalla,[4] Gisela Nogales-Gadea,[5,6] Juan C Rubio,[6,7] Inés García-Consuegra,[6,7] Ana Cabello,[6,7] Margarita Pérez,[1] Susana Teijeira,[8] Irene Vieitez,[8] Carmen Navarro,[6,8] Joaquín Arenas,[6,7] Miguel A Martin,[6,7] Antoni L Andreu[5,6]

EUROMAC- European registry of patients with McArdle disease and very rare muscle glycogenolytic disorders (MGD) with exercise intolerance as the major symptom (PR-MDMGD)

Second Programme of Community action in the field of Health (2008-2013)

Some observations from the Spanish Registry

McArdle Patients (n=239, 137 men, 102 women)

	N	All	Men	Women
Age, years	**239**	**44±18** (9,93)	**43±17** (9,90)	**45±18** (11,93)
Age at which symptoms started % (N)	**178**			
1st decade		**58%**(103)	**60%**(61)	**55%**(42)
2nd decade		**28%**(49)	**27%**(28)	**28%**(21)
3rd decade		**5%**(9)	**3%**(3)	**8%**(6)
≥4th decade		**9%**(17)	**10%**(10)	**9%**(7)
Age of genetic diagnosis % (N)	**210**			
1st decade		**4%**(9)	**5%**(6)	**3%**(3)
2nd decade		**20%**(43)	**21%**(45)	**20%**(18)
3rd decade		**29%**(60)	**31%**(36)	**26%**(24)
≥4th decade		**47%**(98)	**43%**(51)	**51%**(47)

Some observations from the Spanish Registry

McArdle Patients (n=239, 137 men, 102 women)

	N	All	Men	Women
Exercise intolerance, %(n)	**211**	**99,5%**(210)	**99%**(118)	**100%**(92)
Permanent muscle weakness %(n)	**196**	**25%**(49)	**23%**(26)	**28%**(23)
Recurrent episodes of Myoglobinuria, %(n)	**196**	**50%**(98)	**61%**(66)	**40%**(32)
Second wind, %(n)	**86**	**86%**(98)	**85%**(39)	**88%**(35)
Basal serum CK activity %(n)	**168**			
>200U/L		**99%**(166)	**98%**(90)	**100%**(76)
>1000U/L		**79%**(133)	**84%**(77)	**74%**(56)

Some observations from the Spanish Registry

McArdle Patients (n=239, 137 men, 102 women)

	N	All	Men	Women
Disease severity, %(n)	**196**			
Class 0		**8%**(16)	**8%**(9)	**8%**(9)
Class 1		**42%**(82)	**34%**(8)	**53%**(44)
Class 2		**25%**(49)	**35%**(40)	**11%**(9)
Class 3		**25%**(49)	**23%**(26)	**28%**(23)

Disease severity (Martinuzzi Classification):

Class 0: Mild exercise intolerance. No problem with daily life activities.

Class 1: Exercise intolerance, cramps, myalgia, and limitation of acute strenuous exercise, and occasionally in daily life activities; no record of myoglobinuria, no muscle wasting or weakness.

Class 2: Symptoms included in 1, plus recurrent exertional myoglobinuria, moderate restriction in exercise, and limitation in daily life activities.

Class 3: Fixed muscle weakness, with or without wasting and severely limited exercise and most daily life activities.

Some observations from the Spanish Registry

McArdle Patients (n=239, 137 men, 102 women)

Mutation	Allelic frequency	Type of mutation
p.R50X(c.148C>T)	**55%**	**Nonsense**
p.W798R(c.2392T>C)	**11%**	**Missense**
p.G205S(c.613G>A)	**9%**	**Missense**
Others (>20 different mutations)	**25%**	**Diverse**

REGISTRO NACIONAL DE ENFERMEDAD DE McARDLE

(*Hospital 12 de Octubre*, Madrid; *Hospital Val d'Hebron*, Barcelona; and *Hospital Meixoeiro*, Vigo; *Universidad Europea de Madrid*)

- Período: 1998 – Enero 2011.
- 239 casos de origen Caucásico (102 mujeres)
- Edad: 44±18 años (rango: 9 – 93)
- Prevalencia ~1/167,000
- Alelos mutantes *PYGM* 99.6% de los casos
- Existe heterogeneidad en la gravedad de los síntomas, pero hay cuatro características diagnósticas comunes:
  i. 99.5% pacientes crisis aguda de intolerancia al ejercicio (con mioglobinuria recurrente en 50% de los casos)
  ii. 58% pacientes inicia los síntomas en la primera década de vida
  iii. 86% pacientes experimentan el fenómeno 'second wind' repetidamente a lo largo de su vida
  iv. 99% pacientes tienen hiper-CK-emia basal (>200 U/L).
  v. Fracaso renal agudo: muy infrecuente (4%)

- **Los pacientes físicamente activos tienen mayor probabilidad de mejora de su curso clínico (odds ratio: 232.3, 95%CI: 22.5 – 2324.4), y tienen mejor capacidad cardiorrespiratoria (+ 23% VO_2peak , *P*=0.003).**

Heterogeneidad clínica

• Casos inicio tardío de la enfermedad *(Engel, 1963; Felice, et al., 1992; Pourmand, et al., 1983; Wolfe, et al., 2000).*

• 3 casos con debut en el periodo neonatal de una forma infantil fatal *(DiMauro and Hartlage, 1978; Milstein, et al., 1989; Miranda, et al., 1979).*

• 1 caso de muerte súbita *(el-Schahawi, et al., 1997).*

• Doble problema:

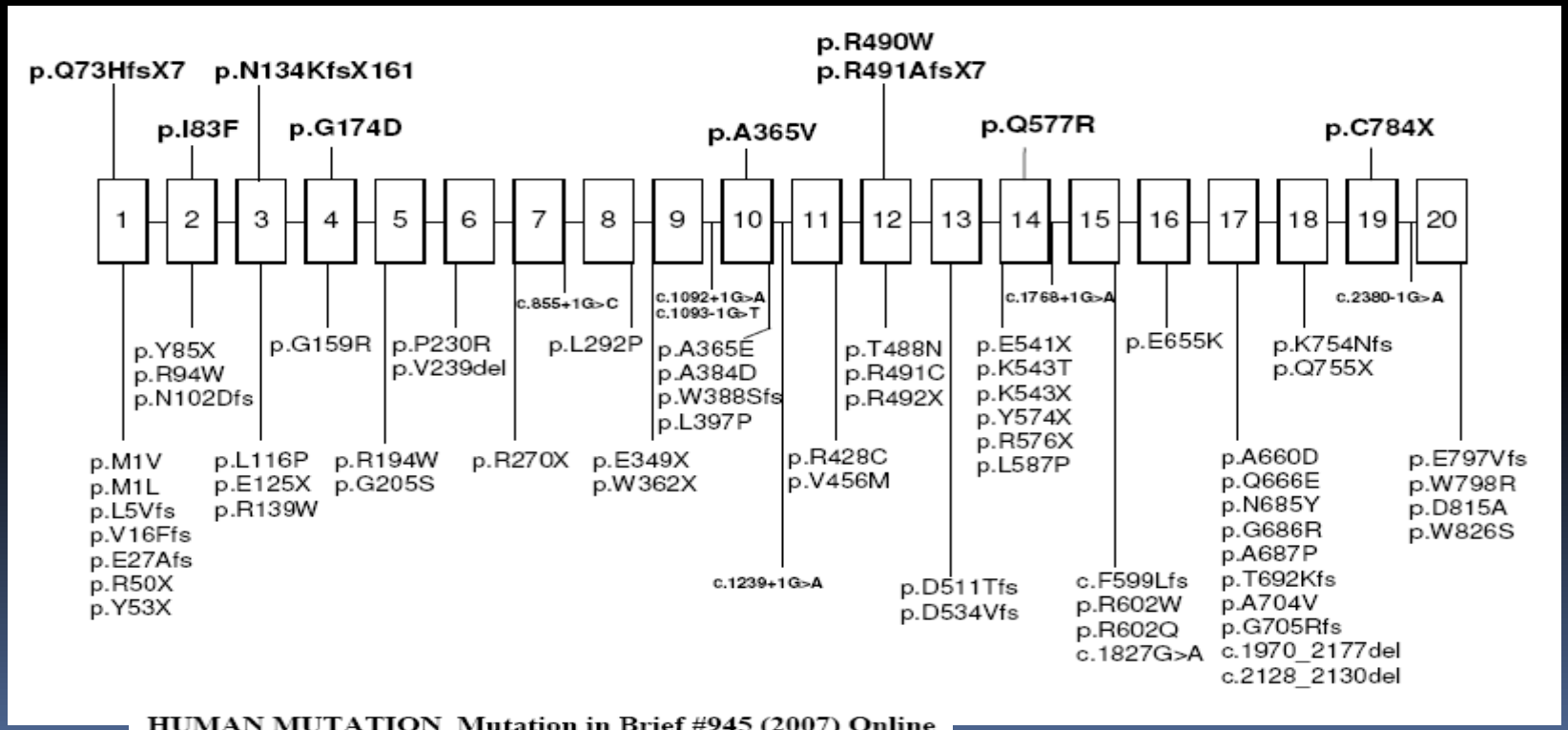
i. MADA *(Rubio, et al., 2000c; Tsujino, et al., 1995a)*,
ii. alteraciones mitocondriales *(Mancuso, et al., 2003; Rubio, et al., 1998)*,
iii. hipertermia maligna *(Isaacs, et al., 1989)*,
iv. miastenia gravis *(Lucia, et al., 2007b)*.

Moduladores fenotípicos

- Gen *ACE,* codifica ECA.
  - Polimorfismo *I/D.* Alelo *I*, ↓ act ECA.
  - Martinuzzi et al, 2003: Mayor incidencia alelo D gen *ACE* en pacientes mayor severidad fenotípica.
- Gen *AMPD1,* codifica MADA.
  - Mutación p.Q12X.
  - Martinuzzi el al, 2003. No asociación.
- Gen *ACTN3*, codifica α-actinina-3.
  - Polimorfismo p.R577X.
- Gen PPARGC1A, Coactivador-1 α del receptor-γ activado por proliferadores de peroxisomas .
  - Polimorfismo p.G482S.

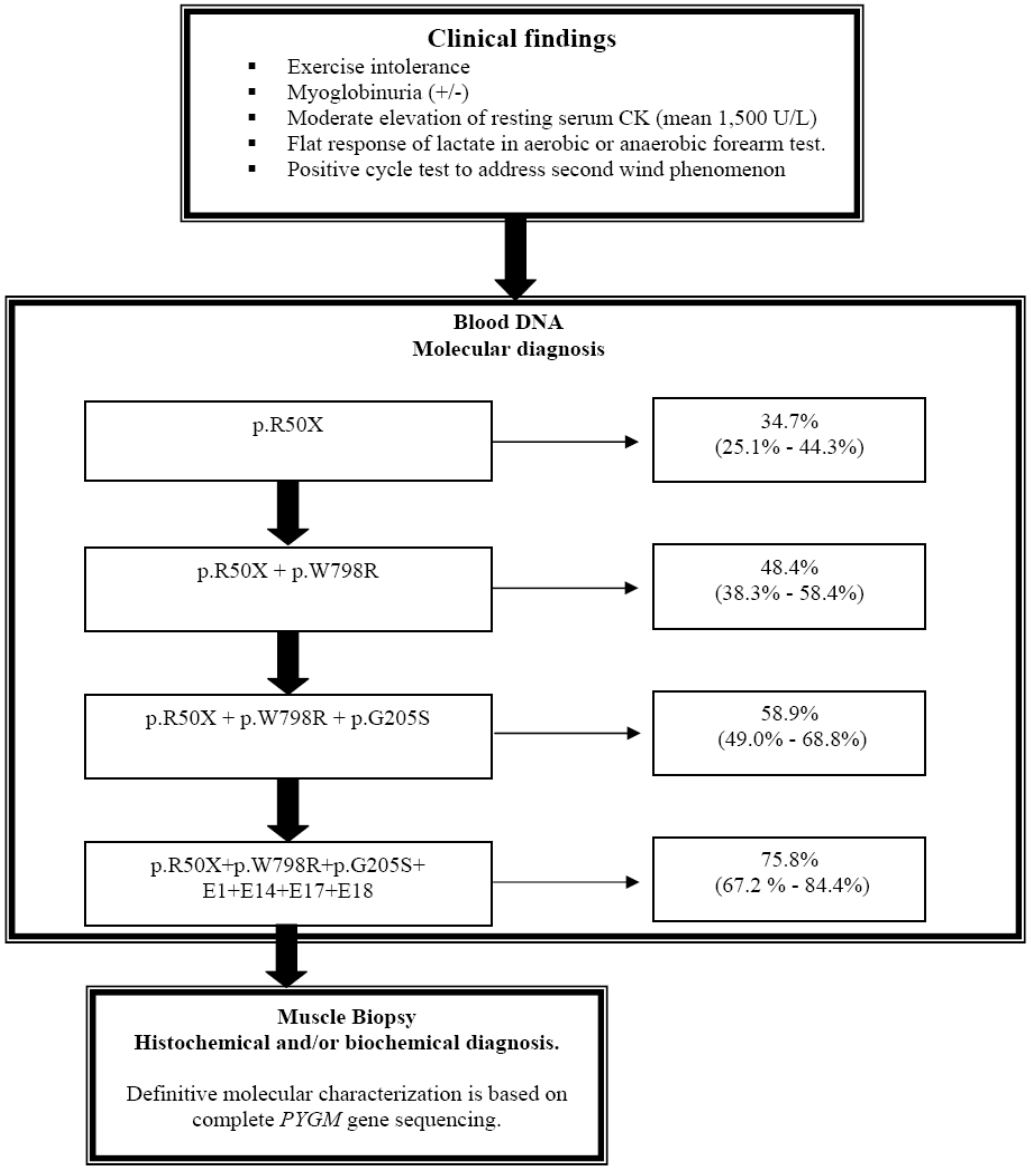
Heterogeneidad molecular

1993, 1ª mutación ⟶ Ene 2013 >130 mutaciones *PYGM.*



HUMAN MUTATION Mutation in Brief #945 (2007) Online

Spanish consortium diagnostic flowchart



HUMAN MUTATION Mutation in Brief #945 (2007) Online

BLOOD
DNA TESTING

Screening PCR-RFLP;
Genotyping real-time PCR

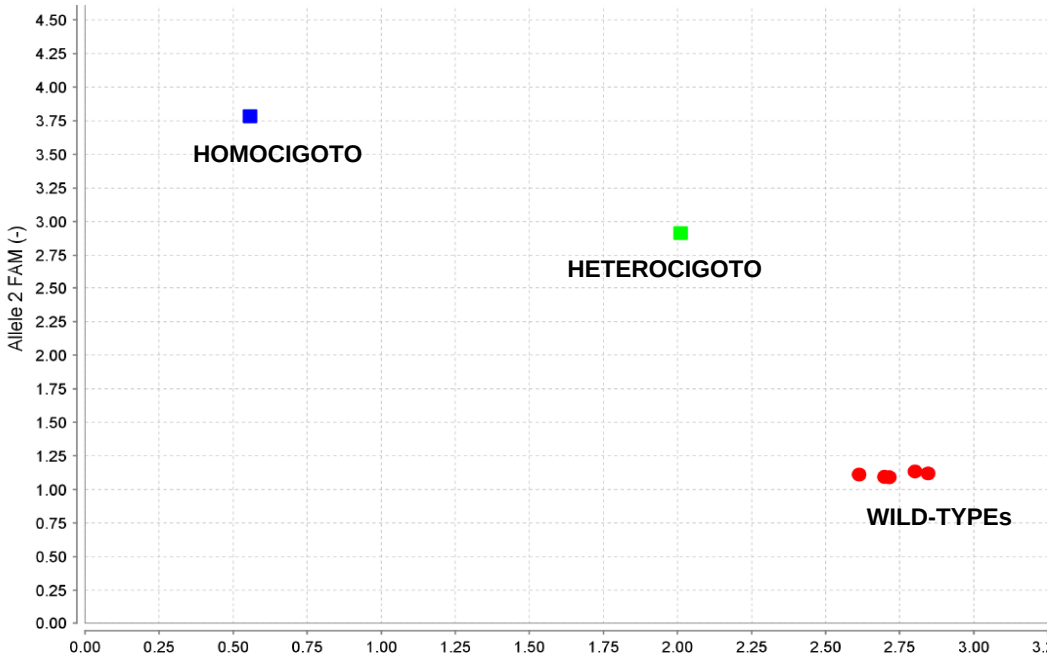
+

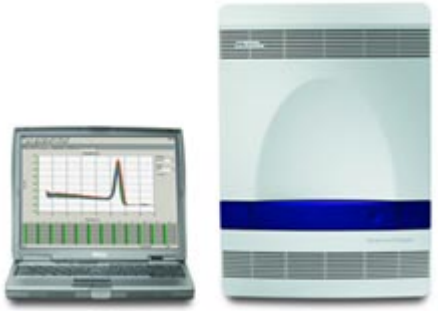
Sequencing
E1, E14, E17, E18

75%

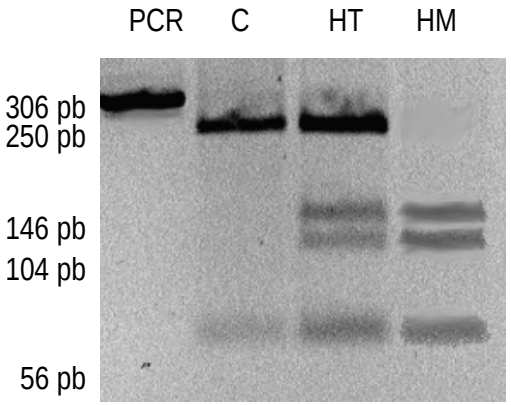
PYGM Genotipado Mutaciones Frecuentes
Discriminación Alélica 'Real Time PCR'

p.R50X-PYGM-McArdle





PCR-RFLP Nla III



REEVALUATION OF THE GENOTYPE-PHENOTYPE RELATIONSHIP

- Reevaluating the functional effect of polymorphisms and “silent” mutations.

- Reinterpreting the genotype-phenotype relationship

- Moving from the genome to the transcriptome.

UNDERSTANDING THE FUNCTIONAL ROLE OF GENOMIC CHANGES AT mRNA AND PROTEIN LEVELS

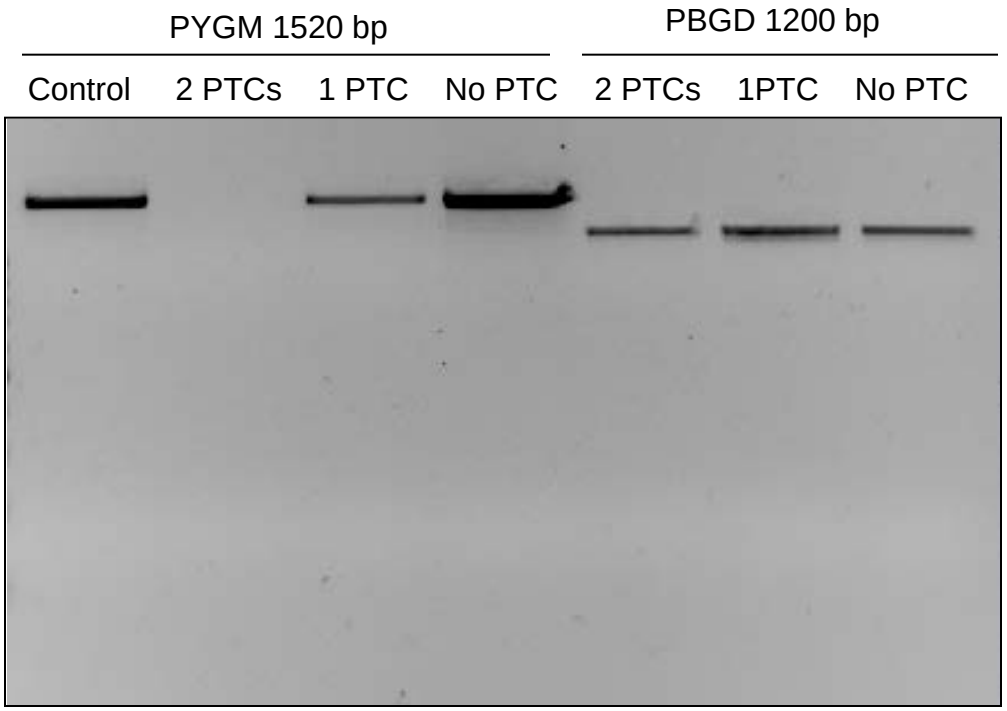
Some patients with the R50X mutation have not detectable mRNA....Why?

Non-Sense Mediated Decay (NMD)

Lynne Elizabeth Maquat, Ph.D

- The transcripts from the majority of mammalian genes are subject to **NMD when they prematurely terminate translation more than ~50-55 nt upstream of the final exon-exon junction**. Therefore, that could explain why disease-associated nonsense codons generally **reduce mRNA abundance** but normal termination codons, which usually reside within the final exon, generally do not.

Transcriptional profile of PYGM: PCR of muscle cDNA



PATIENTS

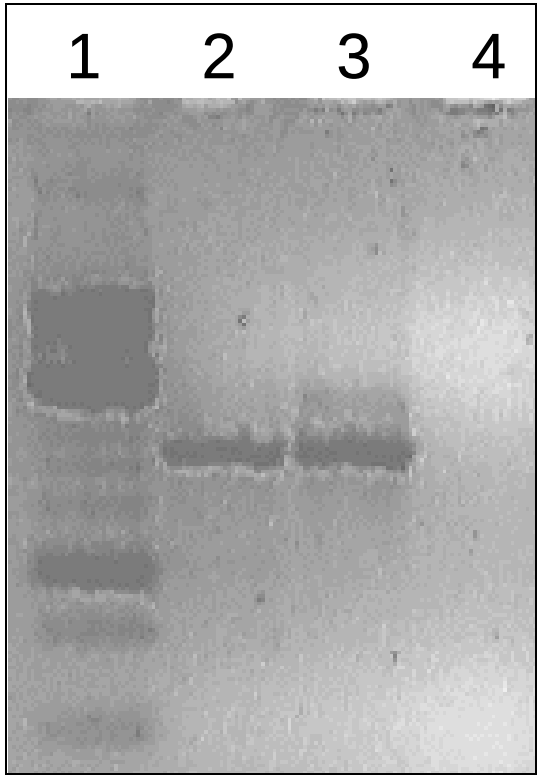
2 PTCs: p.R50X/p.R50X

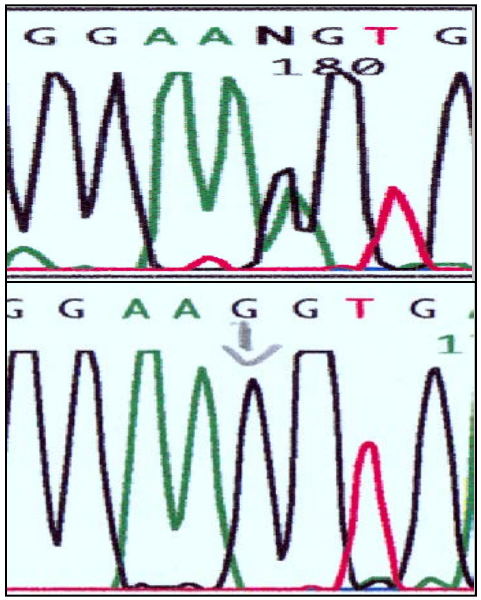
1 PTC: p.R50X/p.R94W

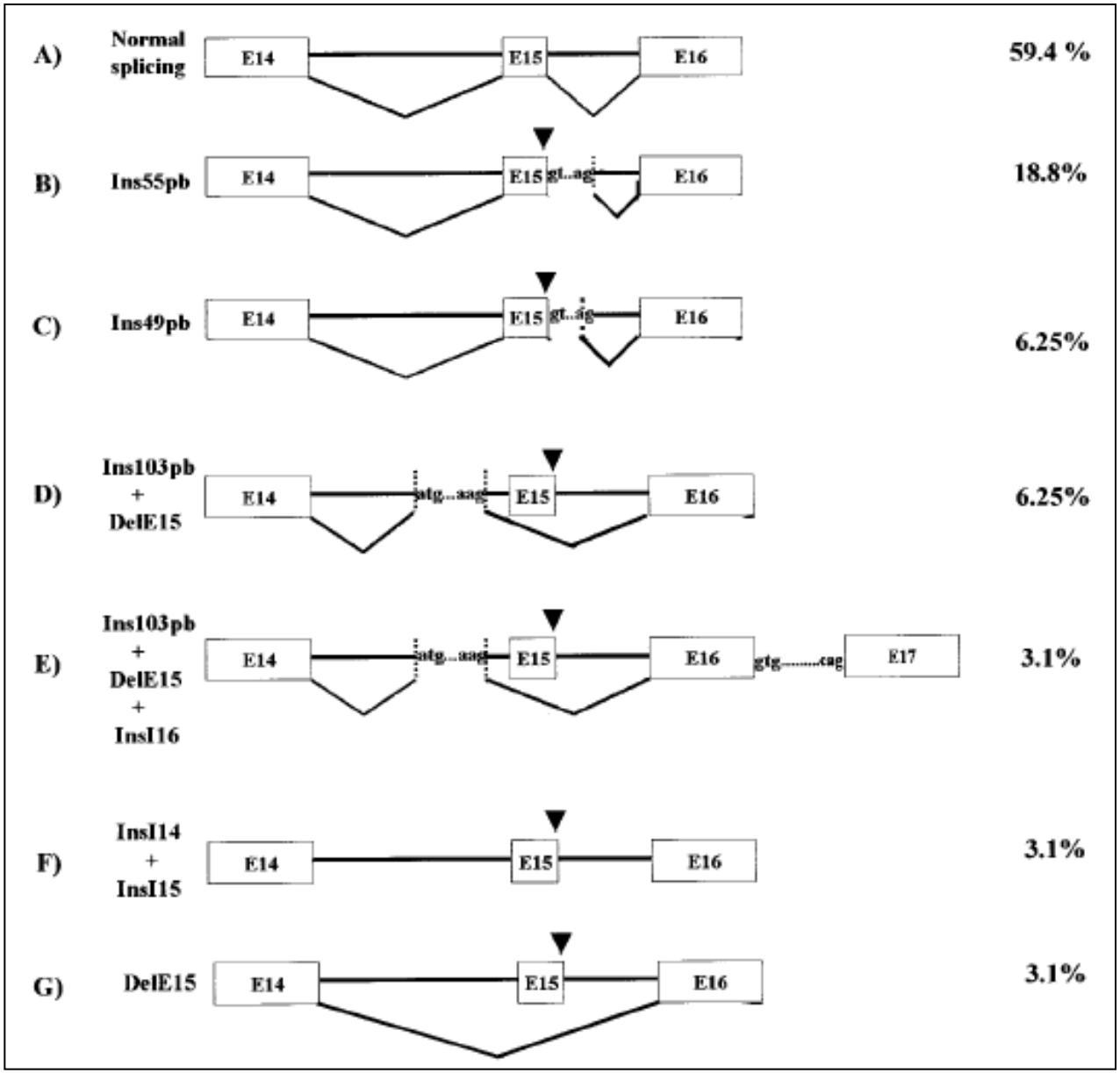
No PTC: p.W798R/p.W798R

Mutaciones “silentes” ???; efectos mRNA

W798R/ ***K609K***

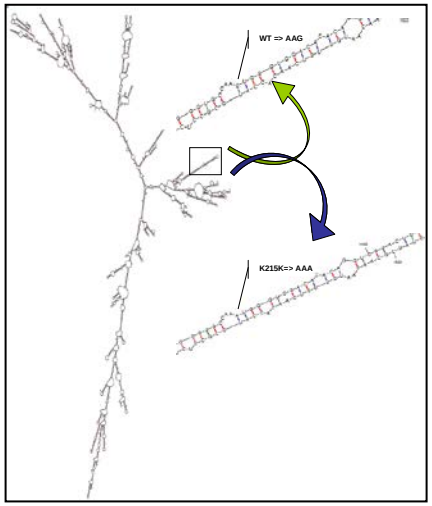


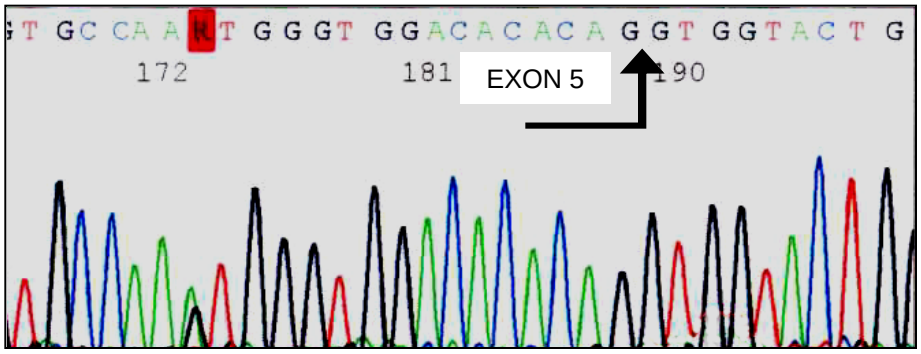



A)
Normal splicing
E14
E15
E16
59.4 %
B)
Ins55pb
E14
E15
gt..ag
E16
18.8%
C)
Ins49pb
E14
E15
gt..ag
E16
6.25%
D)
Ins103pb + DelE15
E14
atg..aag
E15
E16
6.25%
E)
Ins103pb + DelE15 + InsI16
E14
atg..aag
E15
E16
gtg.....cag
E17
3.1%
F)
InsI14 + InsI15
E14
E15
E16
3.1%
G)
DelE15
E14
E15
E16
3.1%

K215K (c.645G>A) (Exón 5)

- **p.R50X + p. K215K (mRNA : 5%)**
- **W798R + p.K215K Una familia:**
  - **Probando: p.R50X+ p.K215K+ p.T488N (cDNA Sangre)**
  - **HIJOS (gDNA sangre):**
    - **2 : p.K215K+ p.T488N (Heterocigosis)**
    - **1 : p.R50X (Heterocigosis)**



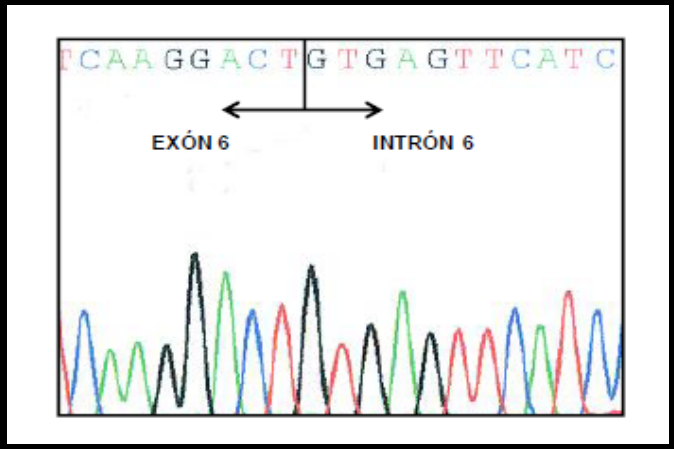


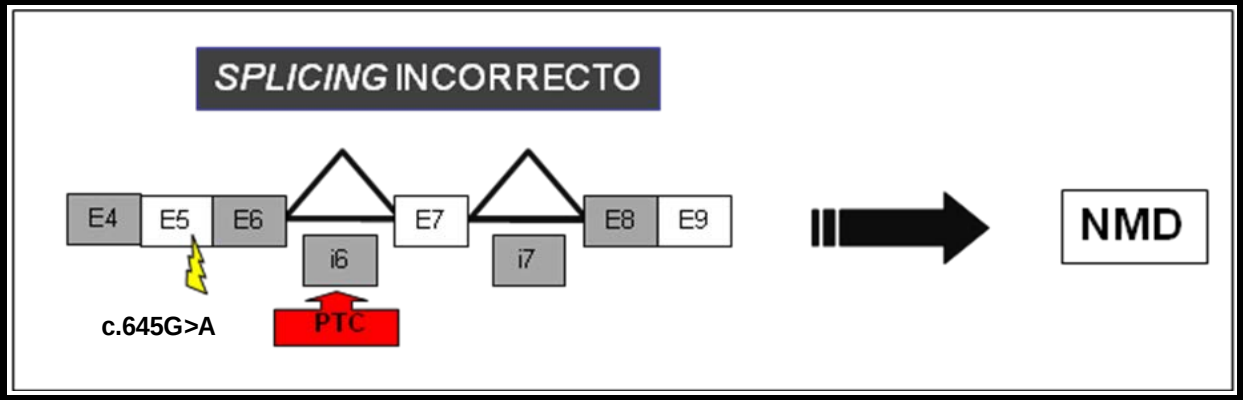
PATOGENICA:

- Estabilidad del mRNA
- ESS?
- ESE?

p.K215K

Electroferograma del ADNc procedente del ARNm del gen *PYGM obtenido a partir de sangre periférica, mostrando la retención del intrón 6 como consecuencia del cambio c.645G>A.*





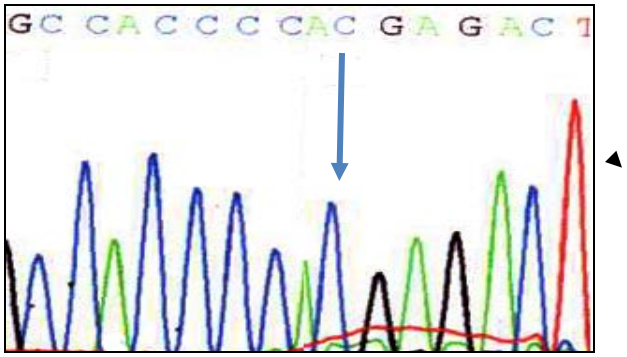
(p.R50X + p.V657_G726del)

ADNc MÚSCULO

c.(1969+214)_(2177+369)del

HOMO

p.R50X WT

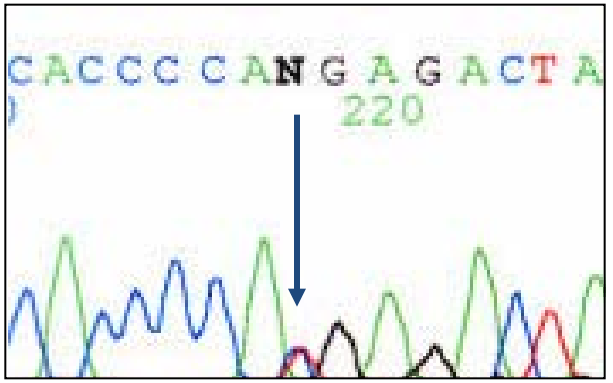


ADNc SANGRE

c.(1969+214)_(2177+369)del

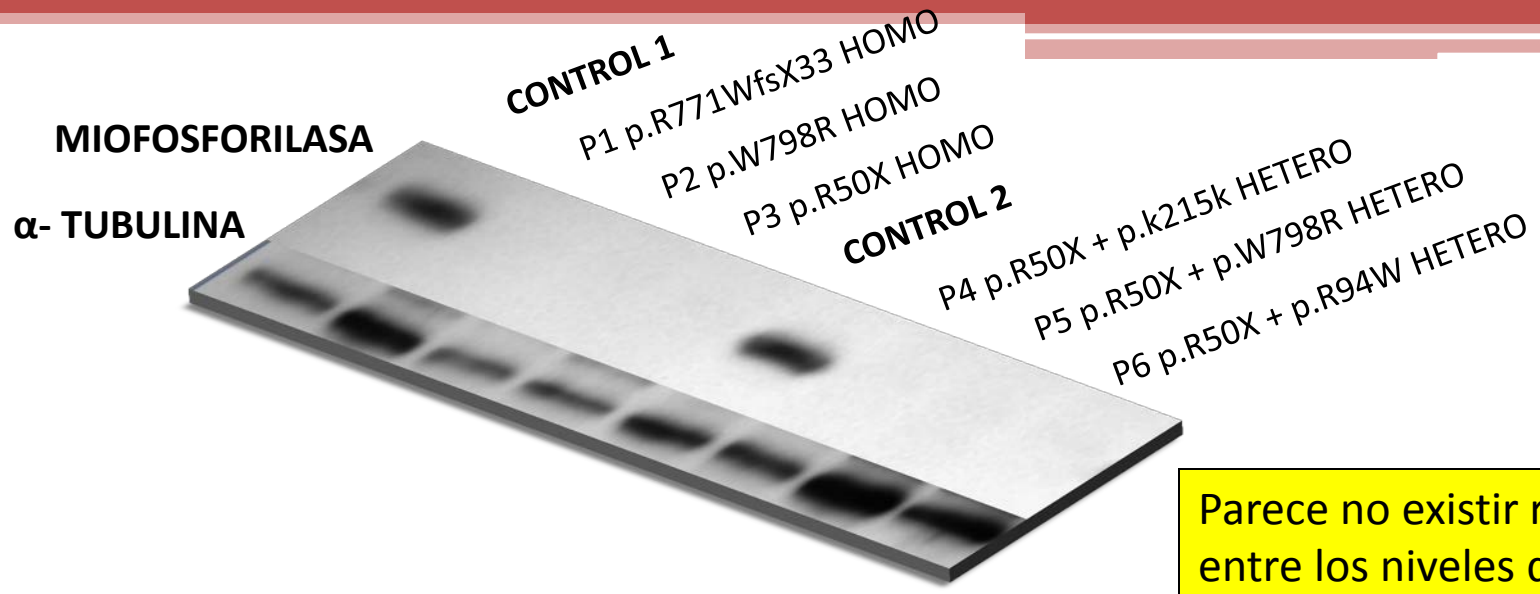
HETEROCIGOSIS

p.R50X

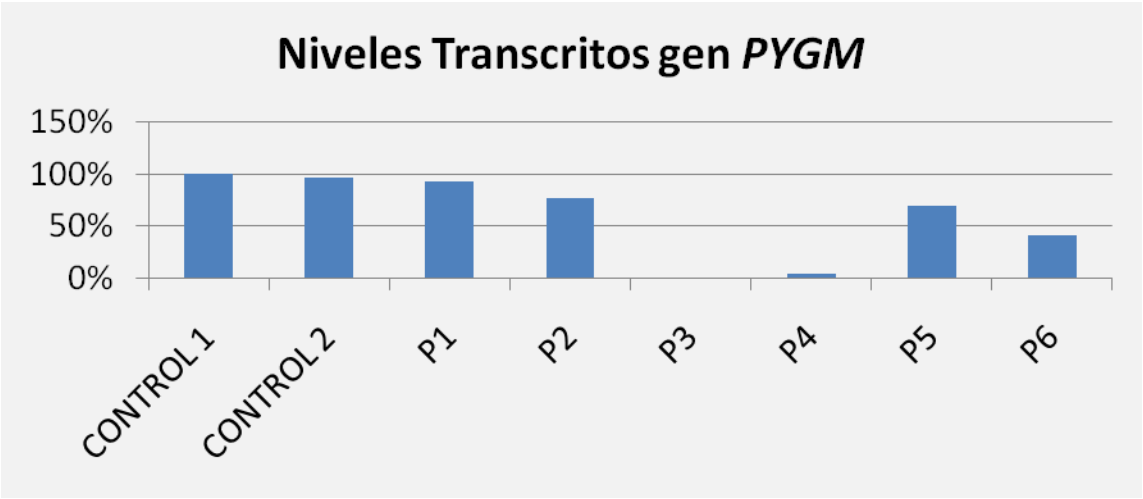


CARACTERIZACION DE LAS ALTERACIONES PROTEÍCAS EN LA ENFERMEDAD DE McARDLE

WESTERN BLOT MIOFOSFORILASA



Parece no existir relación entre los niveles de mensajeros y los niveles de proteína.



CUANTIFICACIÓN TRANSCRITOS DEL GEN *PYGM* MEDIANTE PCR A TIEMPO REAL

Análisis monodimensional

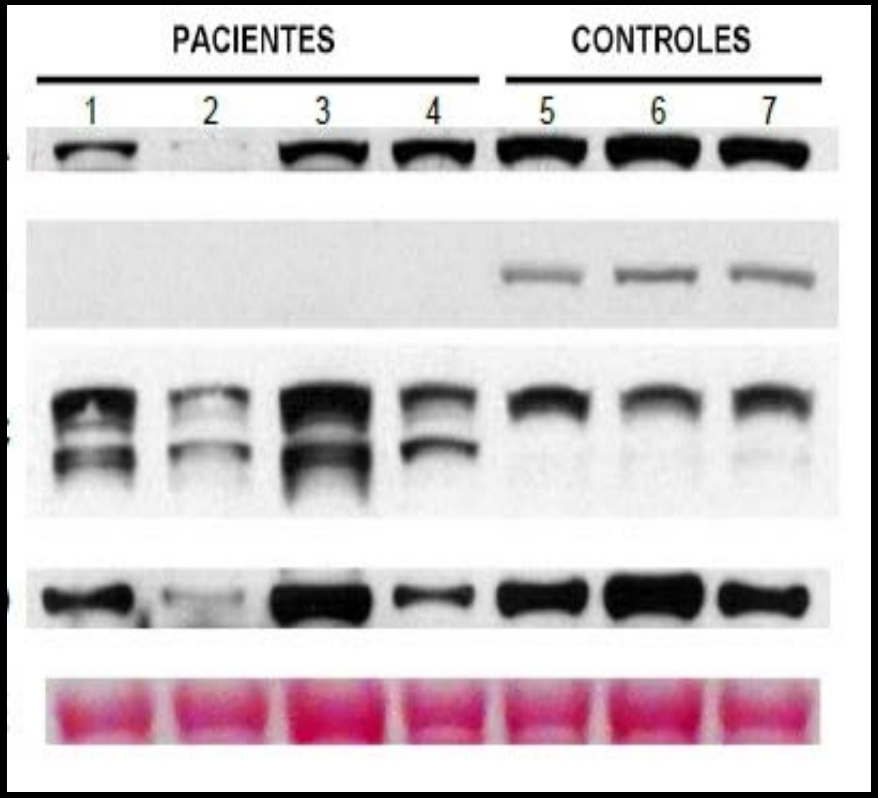
SERCA1
104 KD

GPM
97 KD

GS
84 KD

α- Tubulina
55 KD

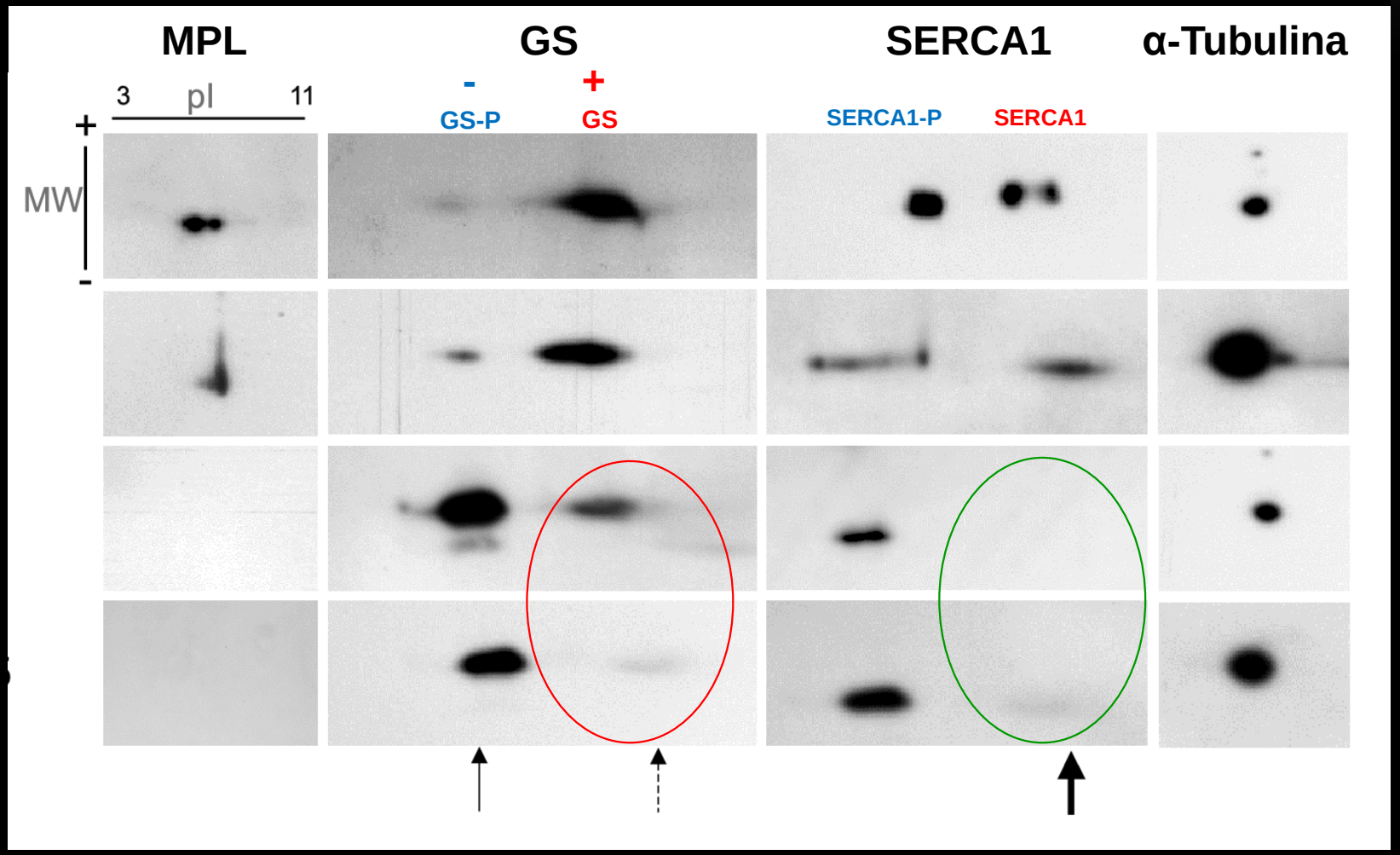
Miosina
217 KD



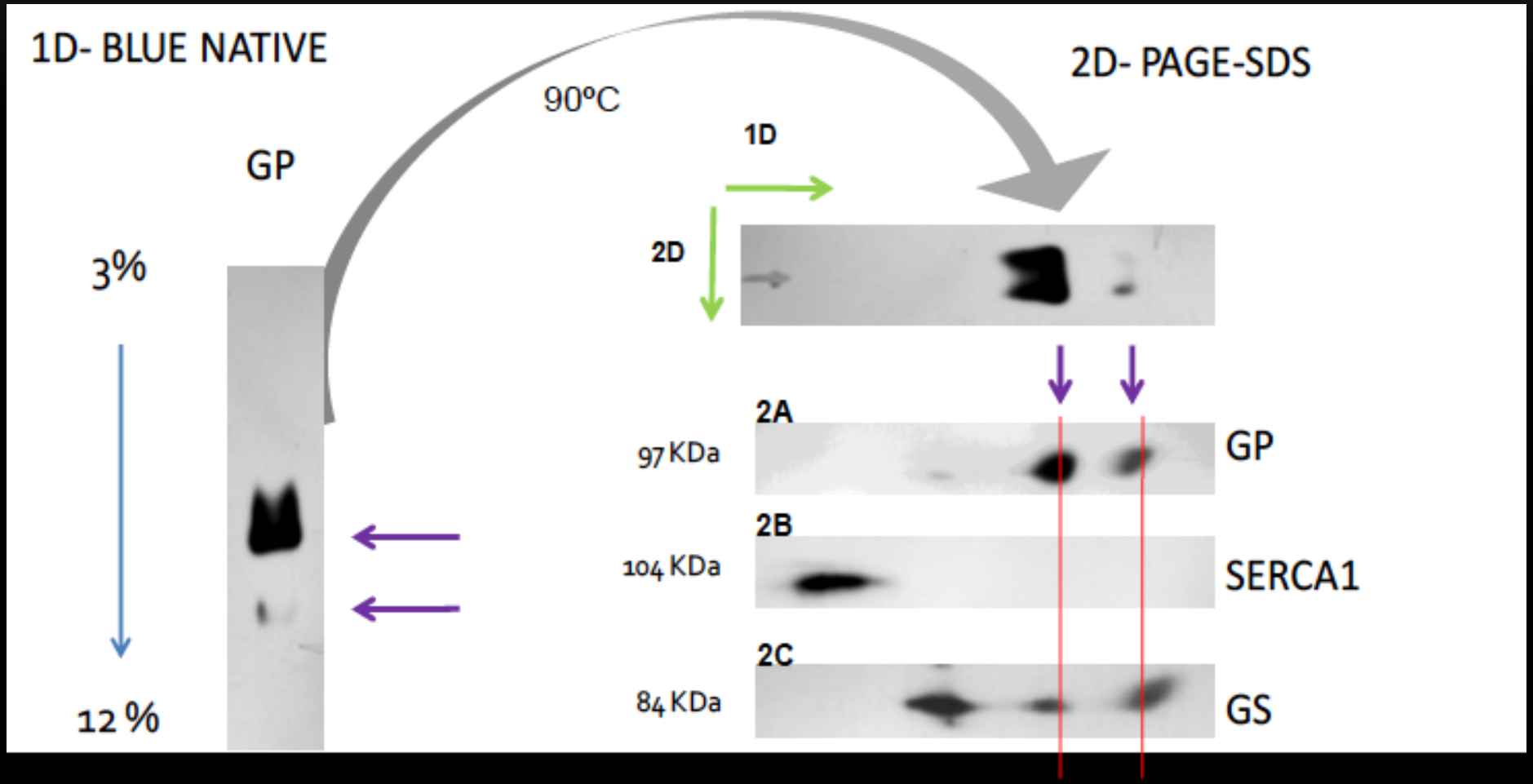
PACIENTES

1. **p.R50X + p.L5VfsX22**
2. **p.W798R HO**
3. **p.R50X HO**
4. **p.R50X + p.A660D**

Análisis Bidimensional - WB



BN- **Colocalizaciones**



La ausencia de GPM en pacientes conlleva la desregulación de puntos clave en el control del metabolismo del músculo esquelético.

i. Una disminución de los niveles y del grado de fosforilación de SERCA1.

ii. Una reducción en la cantidad de GS activa, y un desplazamiento hacia su forma inactiva, lo que sugiere un efecto protector de sobrecarga de glucógeno en el músculo.

iii. Glucógeno sintasa (GS) y GPM parecen colocalizar en el músculo, de tal manera, que la ausencia de GP en los pacientes parece romper el equilibrio de los complejos que forman entre sí, desplazando la GS hacia complejos no activos.

Animal models for McArdle´s disease

Charolais cow

Mutation: R490W
Molecular phenotype:
-No protein
-unknown mRNA levels
Clinical phenotype:
Recurrent myoglobinuria

Merino sheep

Mutation: At the 3'end of intron 19 causes an activation of a cryptic splice within exon 20 generating a frame shift and premature termination of translation.
Molecular phenotype:
No myophosphorylase activity.
Clinical phenotype:
Exercise intolerance

BRAIN
A JOURNAL OF NEUROLOGY

Brain 2012: 135; 2048–2057

Knock-in mice for the R50X mutation in the *PYGM* gene present with McArdle disease

Gisela Nogales-Gadea,[1,2,3,*] Tomàs Pinós,[1,3,*] Alejandro Lucia,[4] Joaquín Arenas,[3,5] Yolanda Camara,[1,3] Astrid Brull,[1,3] Noemí de Luna,[1,3] Miguel A. Martín,[3,5] Elena Garcia-Arumí,[1,3] Ramon Martí[1,3,*] and Antoni L. Andreu[1,3,*]

Why a Knock-in Mouse model?

-The knock-in mouse model present the most frequent mutation in McArdle patients (R.50X).

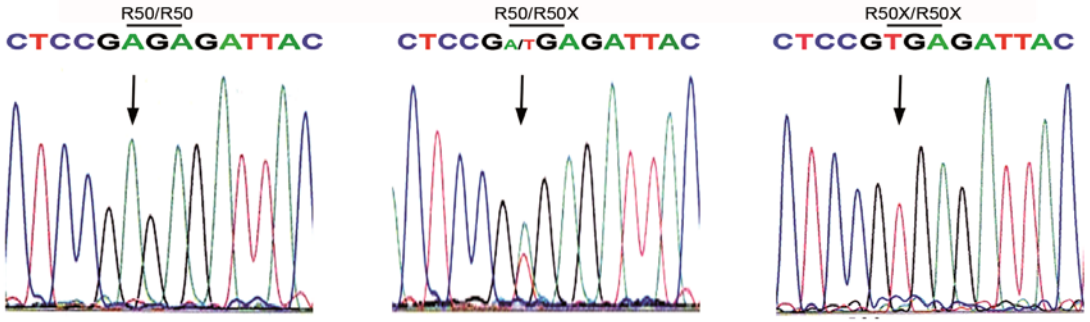
-The knock-in introduction of the R.50X mutation introduces a premature termination codon (PTC) which potentially induces non-sense mediated decay (NMD). It allow us to study NMD processes and possible therapeutic approaches (PTC-124/Ataluren).

-The use of a murine model provides a much more easier manipulation than the other two McArdle mouse models (Charolais cow and Merino sheep). Therapy approaches at the preclinical stage.

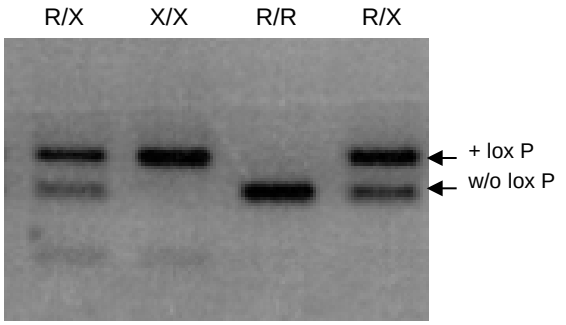
Homozygous mice for R50X

GENOTYPING MICE

by sequencing



by lox p analysis



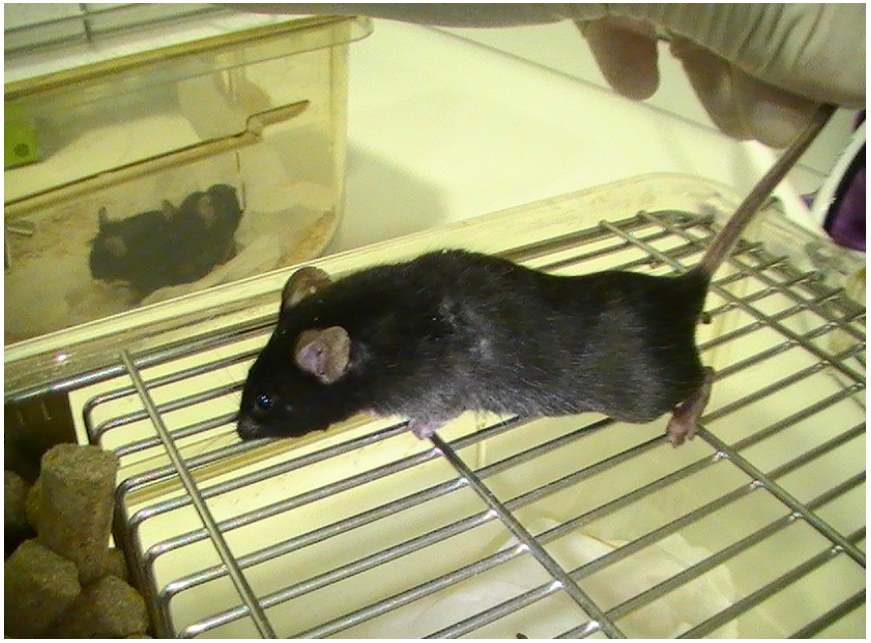
Dimerization domain **p.R50**

```
SHEEP     MSRPLTDQEKRKQISVRGLAGVENVTELKKNFNRHLHFTLVKDRNVATPRDYYFALAYTV 60
CATTLE    MSRPLTDQEKRKQISVRGLAGVENVTELKKNFNRHLHFTLVKDRNVATPRDYYFALAYTV 60
MOUSE     MSRPLSDQDKRKQISVRGLAGVENVSELKKNFNRHLHFTLVKDRNVATPRDYYFALAHTV 60
RABBIT    MSRPLSDQEKRKQISVRGLAGVENVTELKKNFNRHLHFTLVKDRNVATPRDYYFALAHTV 60
HUMAN     MSRPLSDQEKRKQISVRGLAGVENVTELKKNFNRHLHFTLVKDRNVATPRDYYFALAHTV 60
SALMON    MPKPLTDQEKRKQISVRGLAGVENVSDLKTNFNRHLHFTLVKDRNVATKRDYYFALANTV 60
          *.:**:**:******************::**.****************** ******** **
```

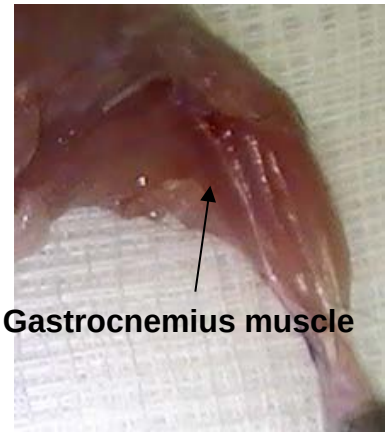
98% protein homology between human and mouse

82% protein homology between human and salmon

MUSCLE EXTRACTION



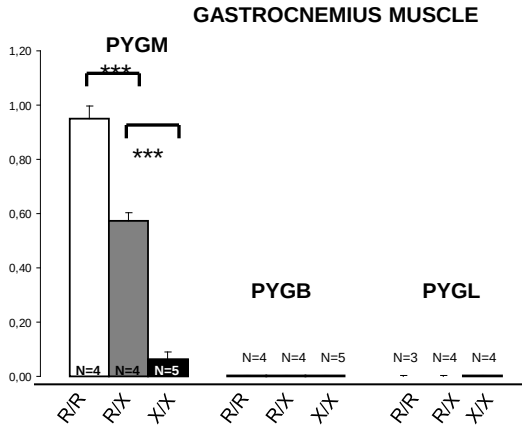
HOMOZYGOUS R50X MOUSE



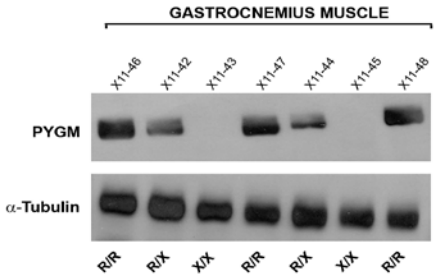
HOMOZYGOUS R50X MOUSE

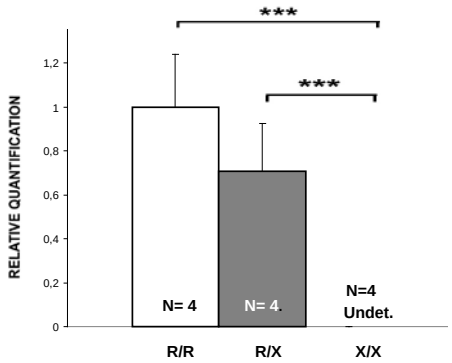
MOLECULAR PHENOTYPE

- **mRNA levels**

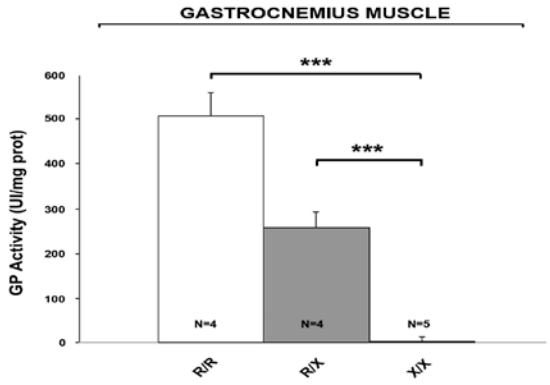


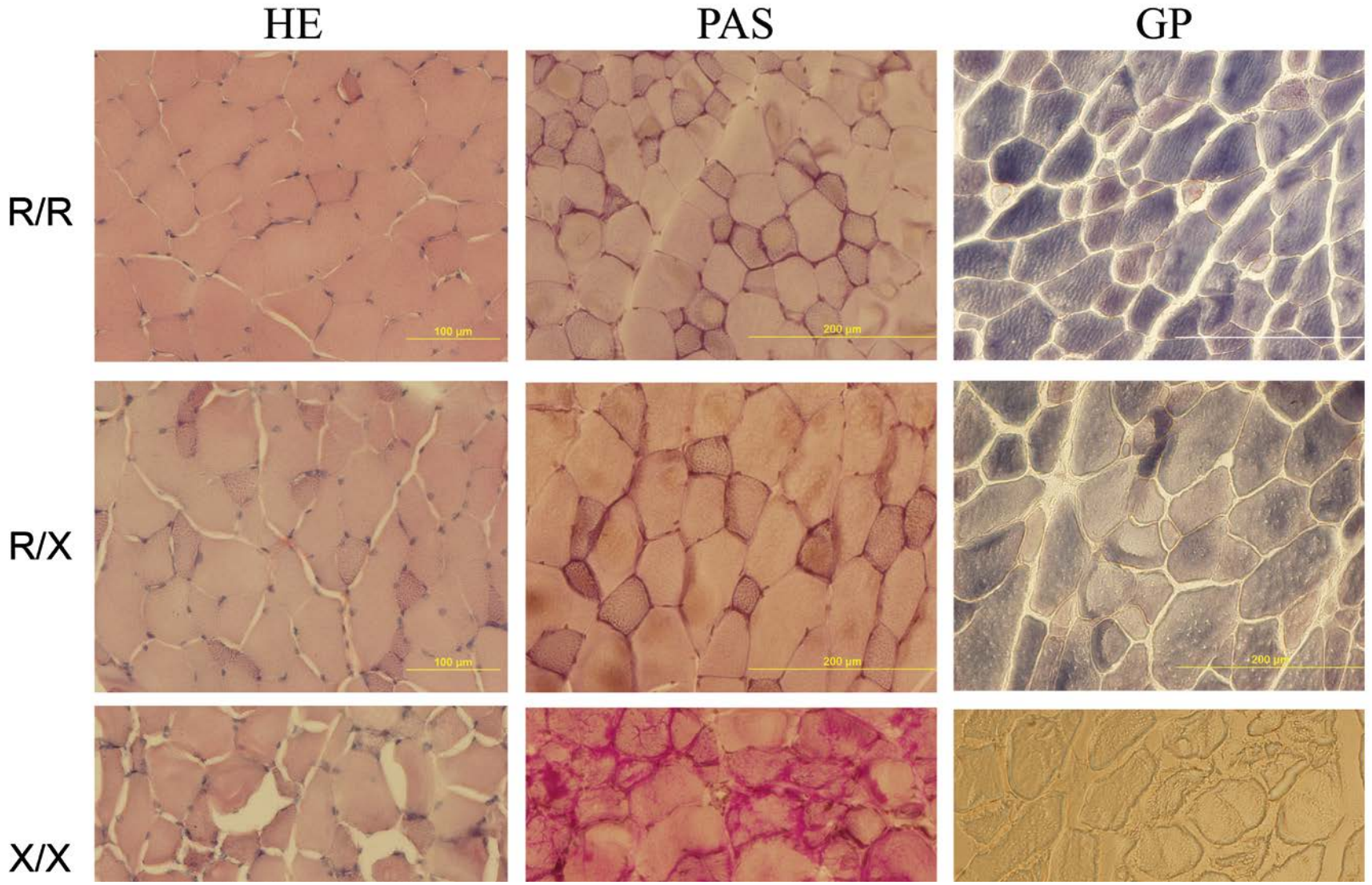
- **Protein levels**



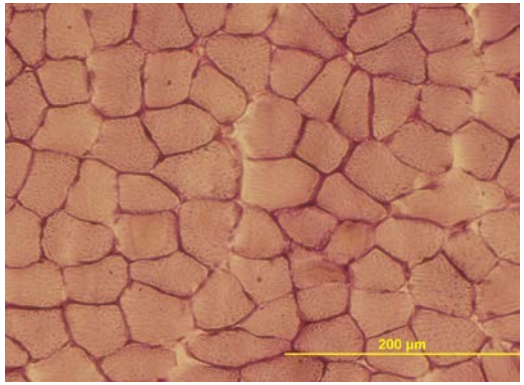
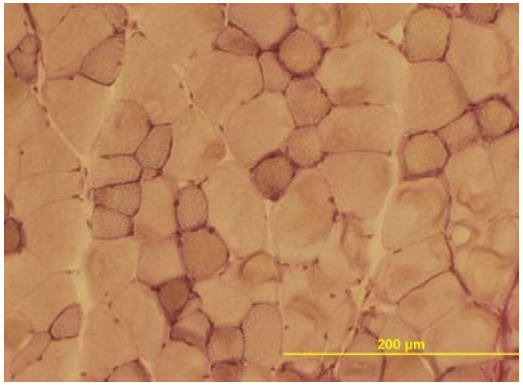
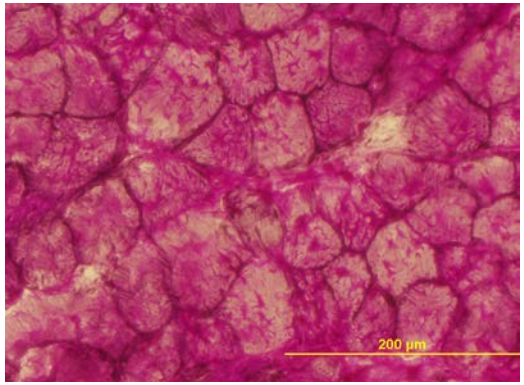
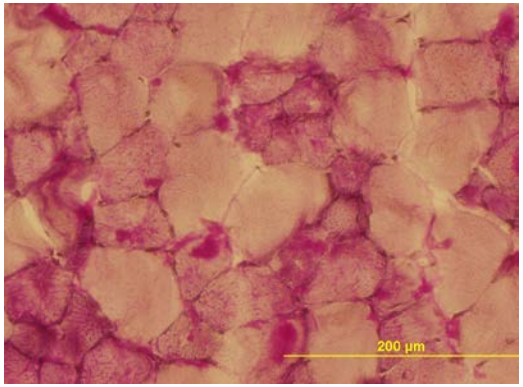


- **GP activity levels**

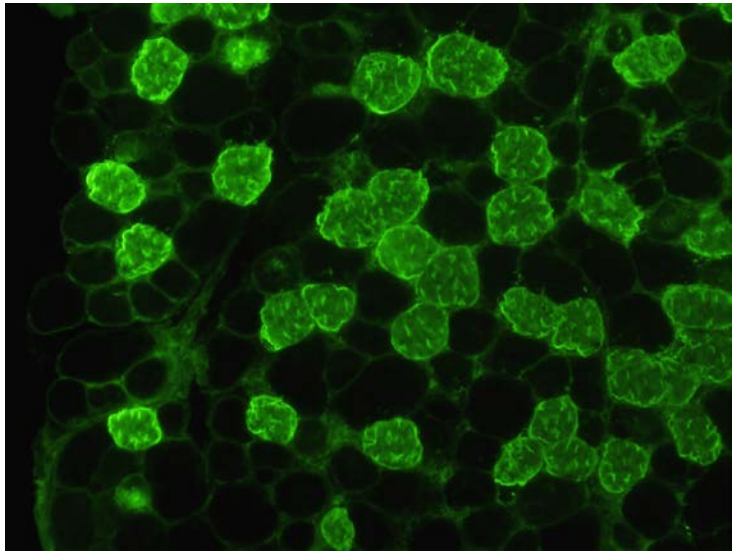



HE
PAS
GP
R/R
R/X
X/X
100 µm
200 µm
100 µm
200 µm
200 µm

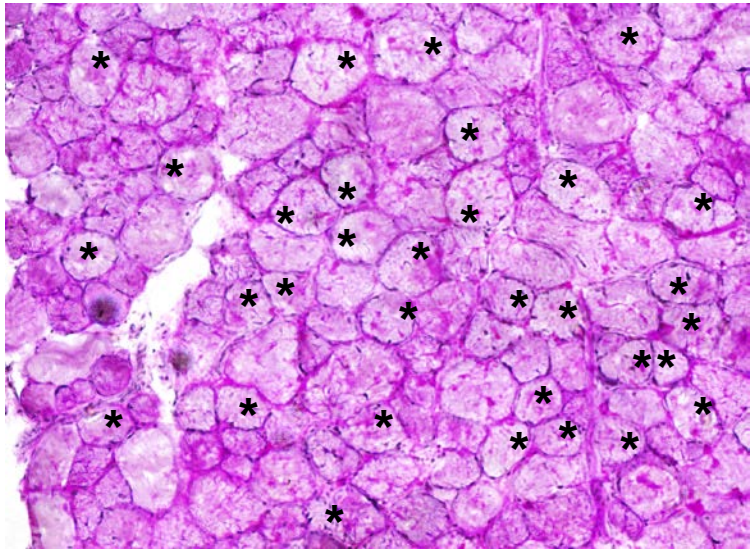
PAS STAINING IN DIFFERENT MUSCLES

	SOLEUS	BICEPS	GASTROCNEMIUS
R/R			
X/X			
	oxidative	glycolitic	mixed

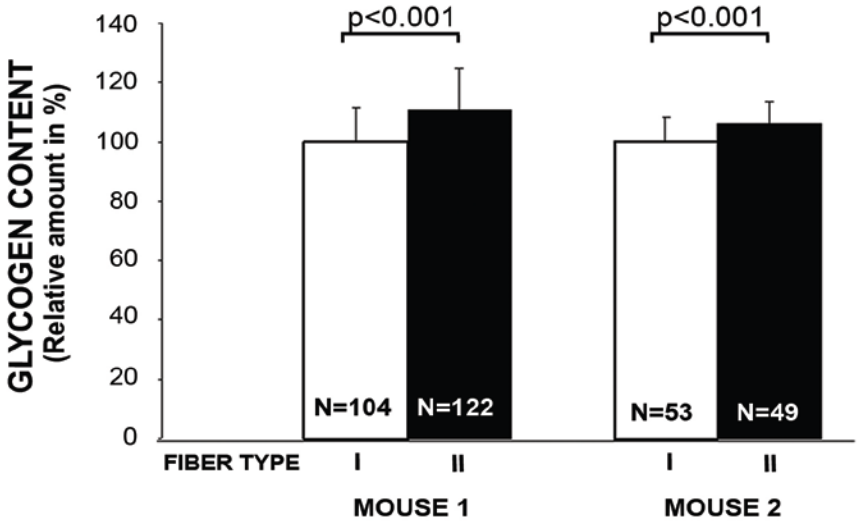
HIGHER GLYCOGEN ACCUMULATION IN TYPE II FIBERS



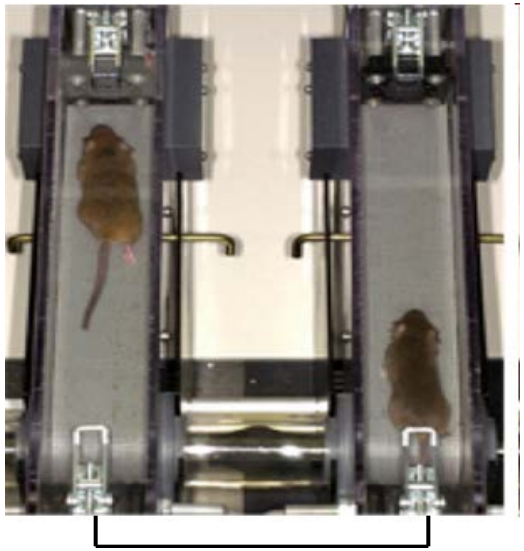
MHC type I



PAS-staining



TREADMILL EXPERIMENT



EXPERIMENT DESIGN:

Inclination : 45º

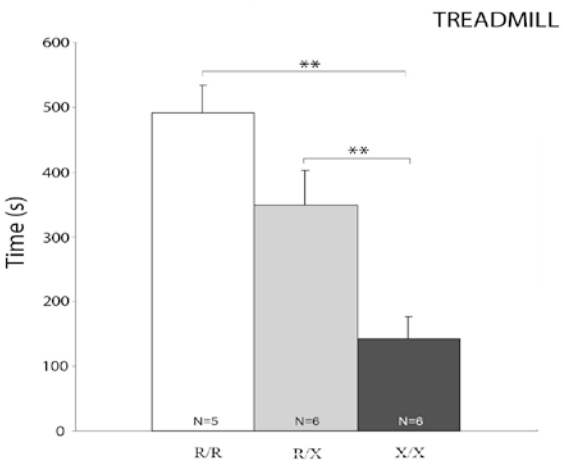
Speed:	0 cm/seg	15 cm/seg	30 cm/seg	40 cm/seg	50 cm/seg	60 cm/seg	**STOP**
Time :	1 min	2 min	2 min	2 min	2 min	2 min	2 min

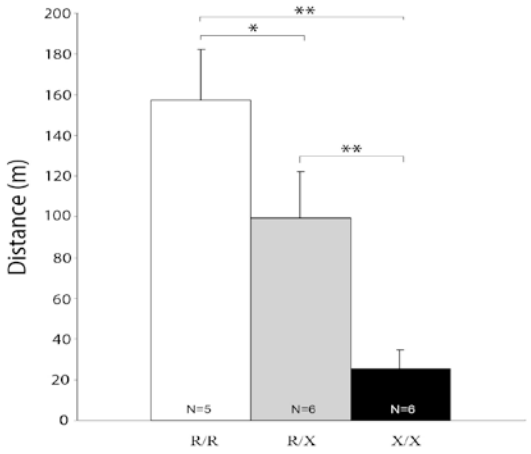
Stop: A) When more than 50% of time in the electric grid or **B)** more than 5 consecutive seconds in the electric grid

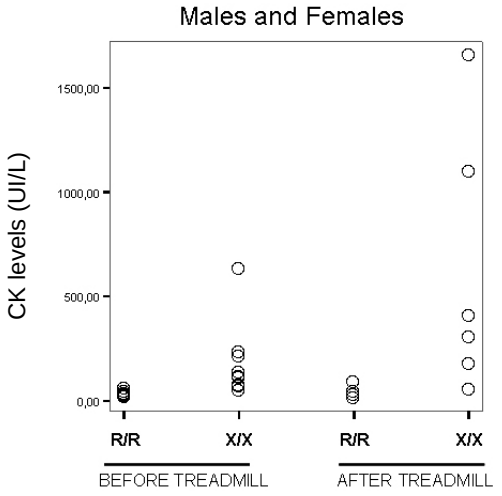
TREADMILL EXPERIMENT: RESULTS

WILD-TYPE (R/R)

HOMOZYGOUS (X/X)



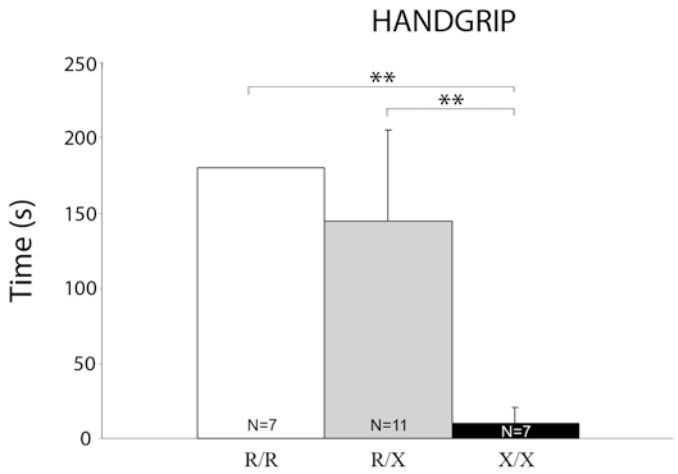




HANDGRIP EXPERIMENT

WILD-TYPE (R/R)

HOMOZYGOUS (X/X)



This model offers...

- **Treatment:** As the mice model presents a similar phenotype to the human disease, it represents a good opportunity to develop and perform studies of potential treatments for McArdle disease at preclinical stages.

- **PTC-targeted drugs:** It is an excellent model to develop pharmacological approaches based on the investigation of new drugs designed to enable the formation of a functioning protein in patients with genetic disorders due to a nonsense mutation.