

**Hospital Universitario
Ramón y Cajal**

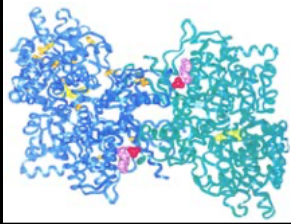


Comunidad de Madrid

**Enfermedades Musculares
en la
Infancia y Adolescencia (X)**

VIERNES 22 de Marzo

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



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

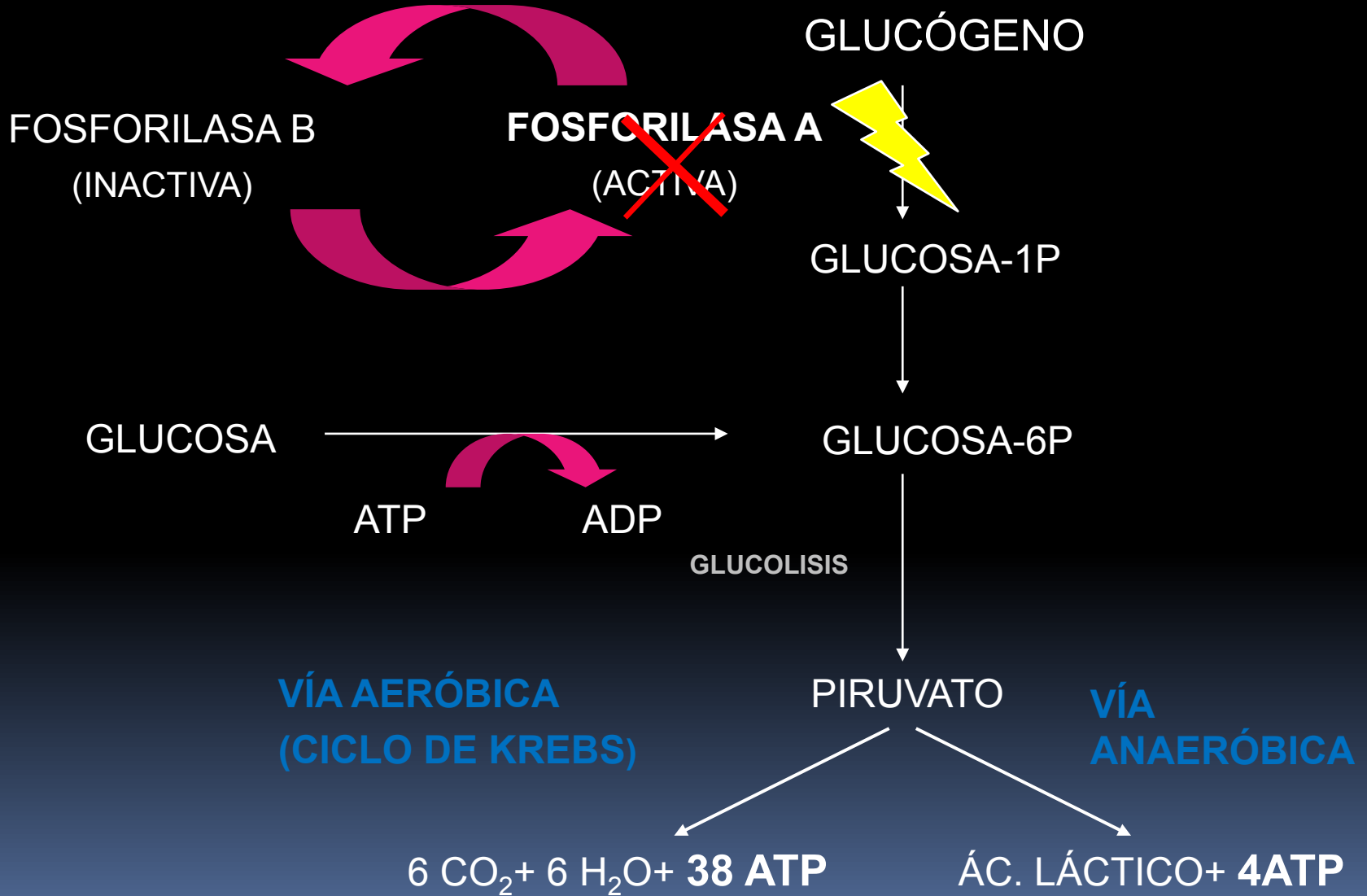
MIGUEL ANGEL MARTIN CASANUEVA
LABORATORIO DE ENFERMEDADES MITOCONDRIALES Y NEUROMETABÓLICAS
S. BIOQUÍMICA CLÍNICA. i+12.
HOSPITAL UNIVERSITARIO 12 DE OCTUBRE. MADRID

Spanish Consortium for McArdle's disease



Enfermedad de McArdle

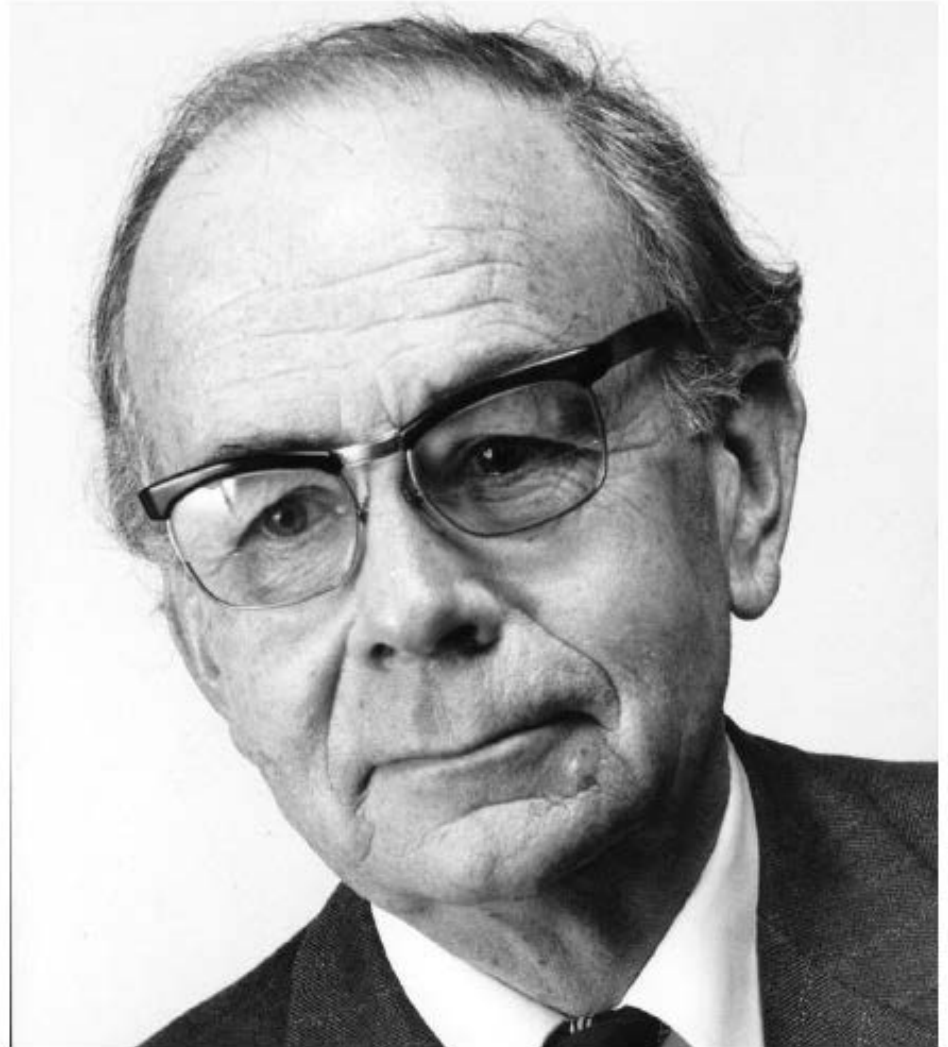
Función Miofosforilasa



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 $\approx$ 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 década, >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.

RESEARCH PAPER

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

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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 $\sim 1/167,000$
- 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_2 peak , $P=0.003$).**

Heterogeneidad clínica

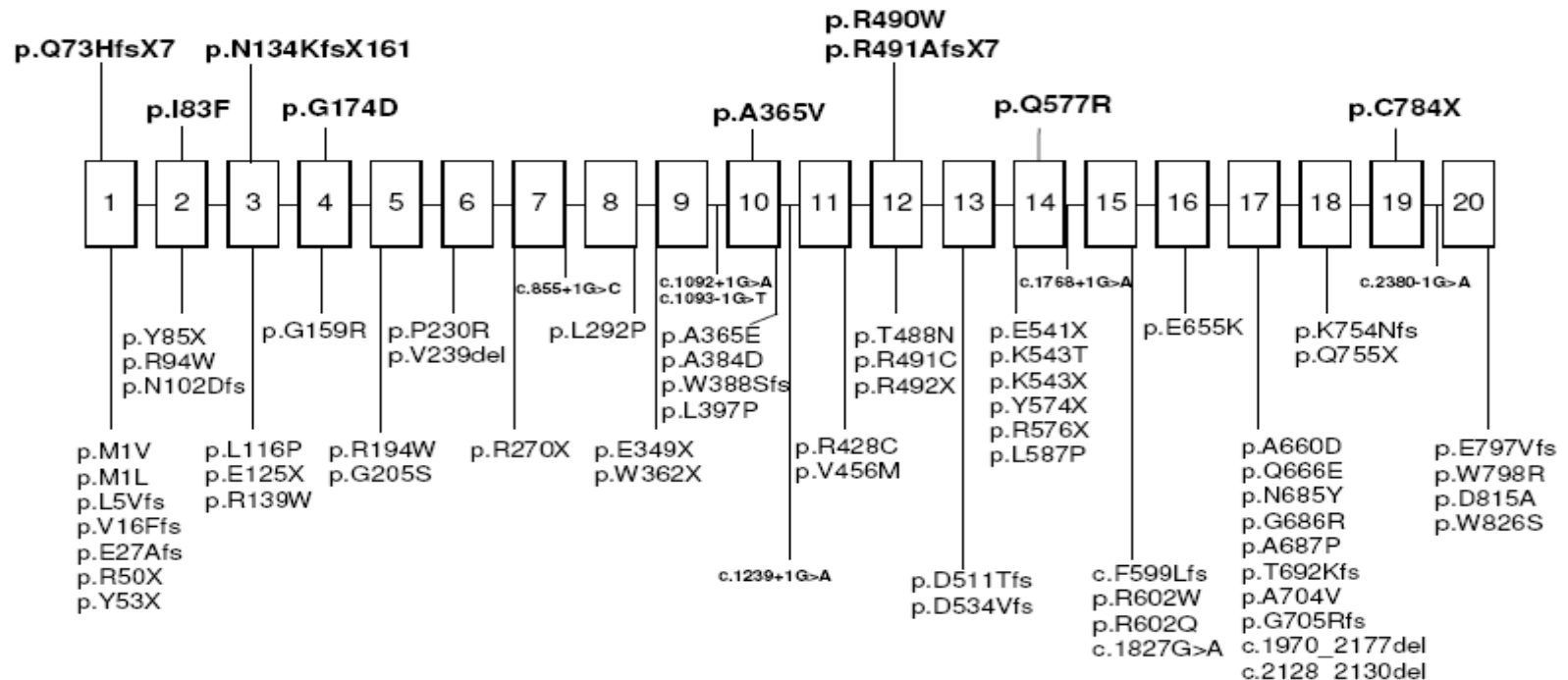
- Casos inicio tardío de la enfermedad (*Engel, 1963; Felice, et al., 1992; Pourmand, et al., 1983; Wolfe, et al., 2000*).
- 3 casos con debut en el periodo neonatal de una forma infantil fatal (*DiMauro and Hartlage, 1978; Milstein, et al., 1989; Miranda, et al., 1979*).
- 1 caso de muerte súbita (*el-Schahawi, 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

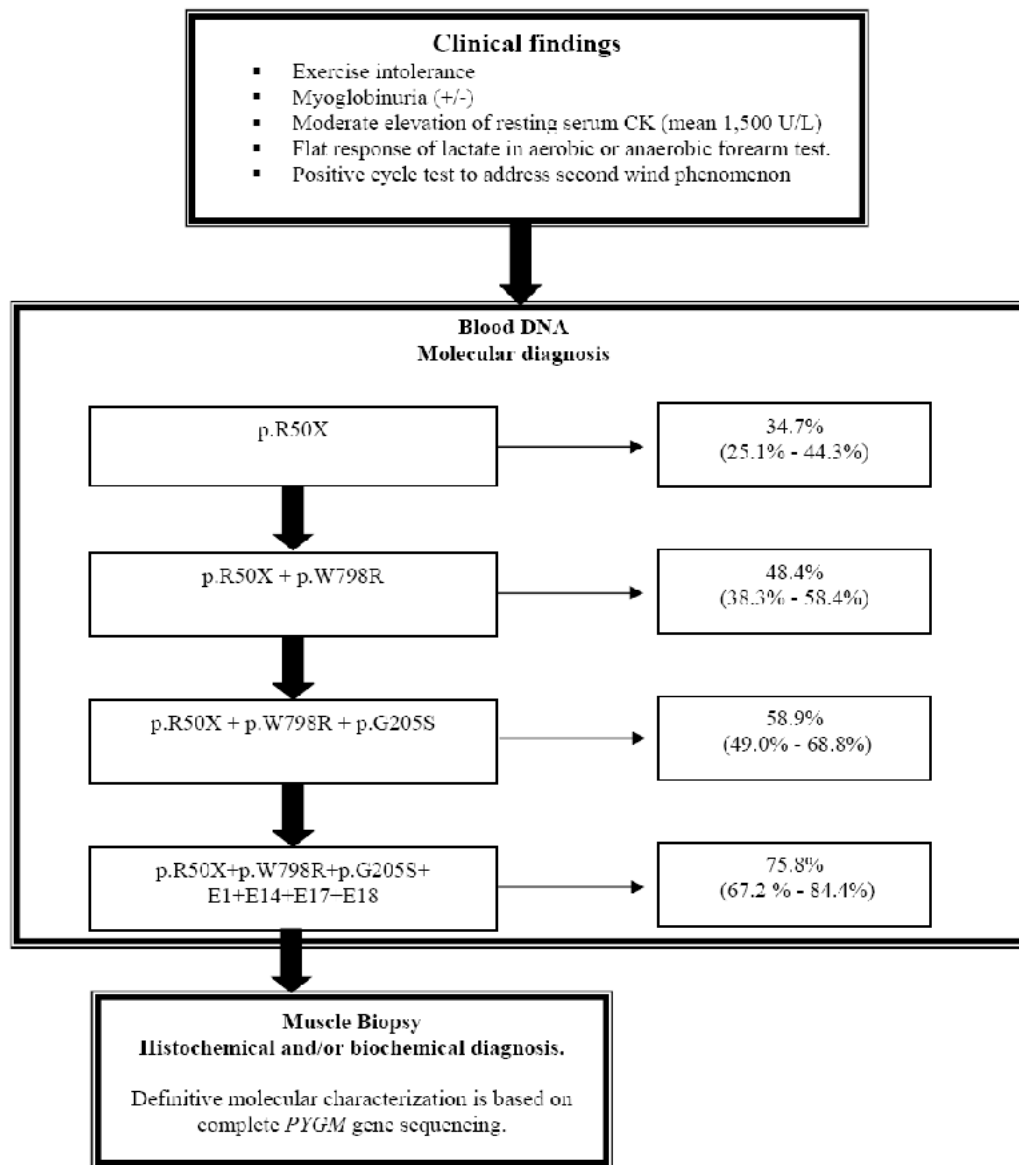
- Gen *ACE*, codifica ECA.
 - Polimorfismo I/D. Alelo I, ↓ act ECA.
 - Martinuzzi et al, 2003: Mayor incidencia alelo D gen *ACE* en pacientes mayor severidad fenotípica.
- Gen *AMPD1*, codifica MADA.
 - Mutación p.Q12X.
 - Martinuzzi et al, 2003. No asociación.
- Gen *ACTN3*, codifica α -actinina-3.
 - 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*.



Spanish consortium diagnostic flowchart



**BLOOD
DNA TESTING**

Screening PCR-RFLP;
Genotyping real-time PCR

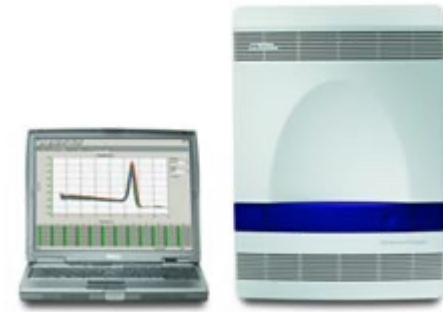
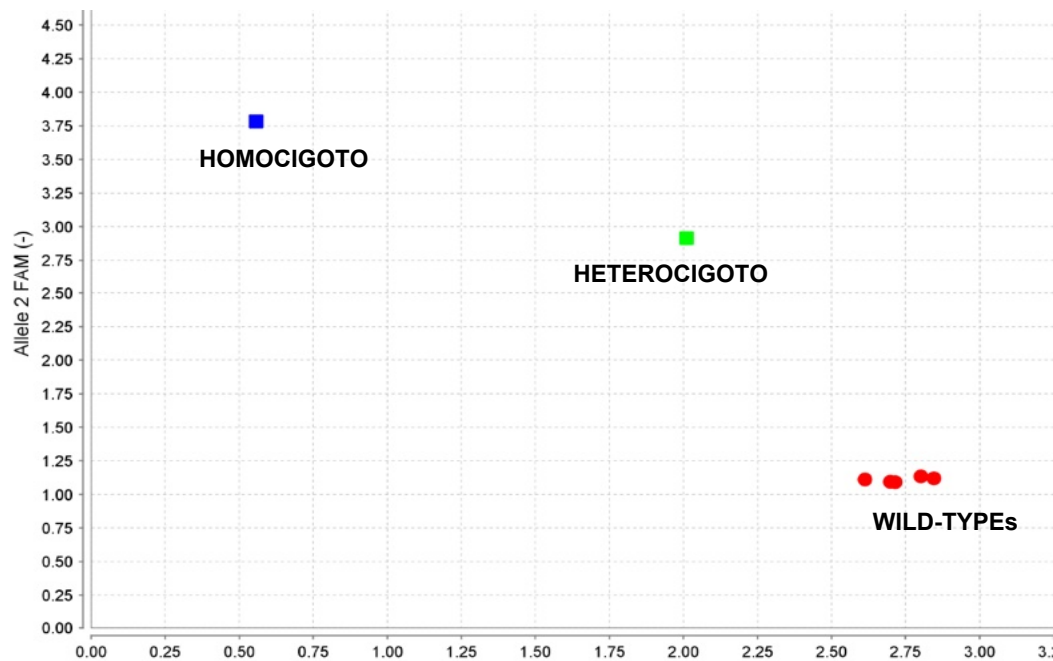
+
Sequencing
E1, E14, E17, E18

75%

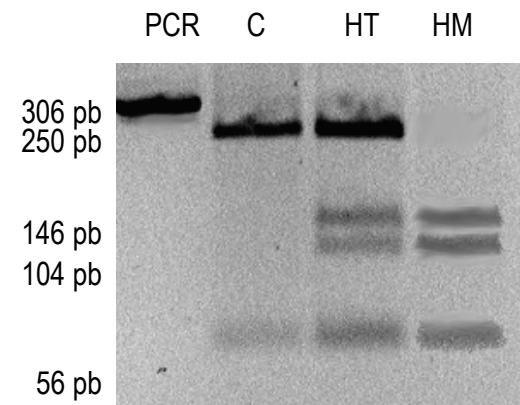
PYGM Genotipado Mutaciones Frecuentes

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)



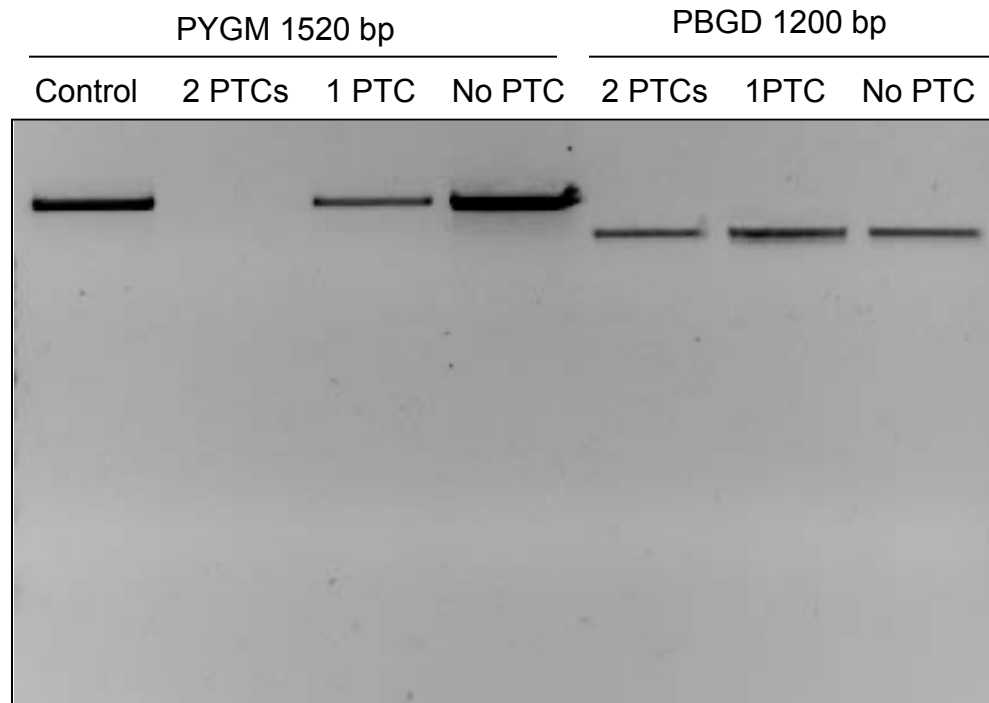
Maquat Lab
normal & disease-associated RNA decay



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

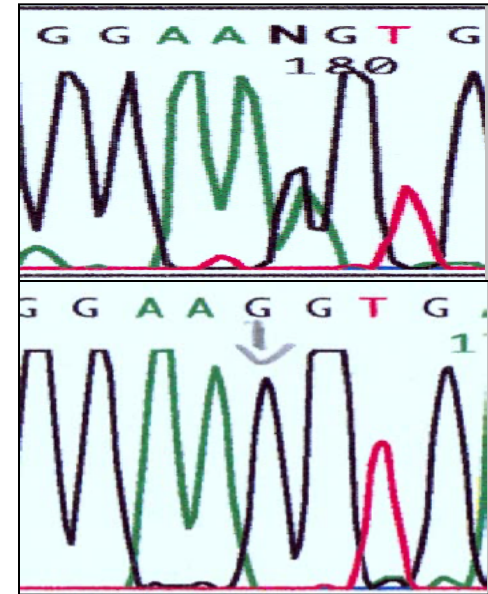
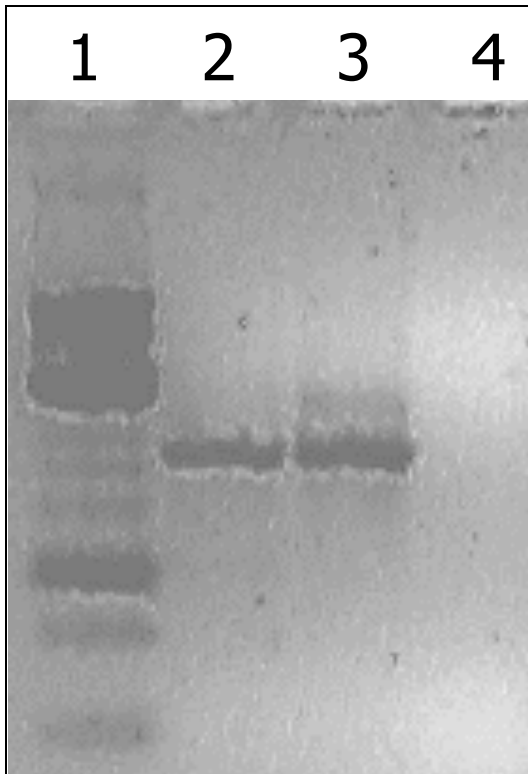
1 PTC: p.R50X/p.R94W

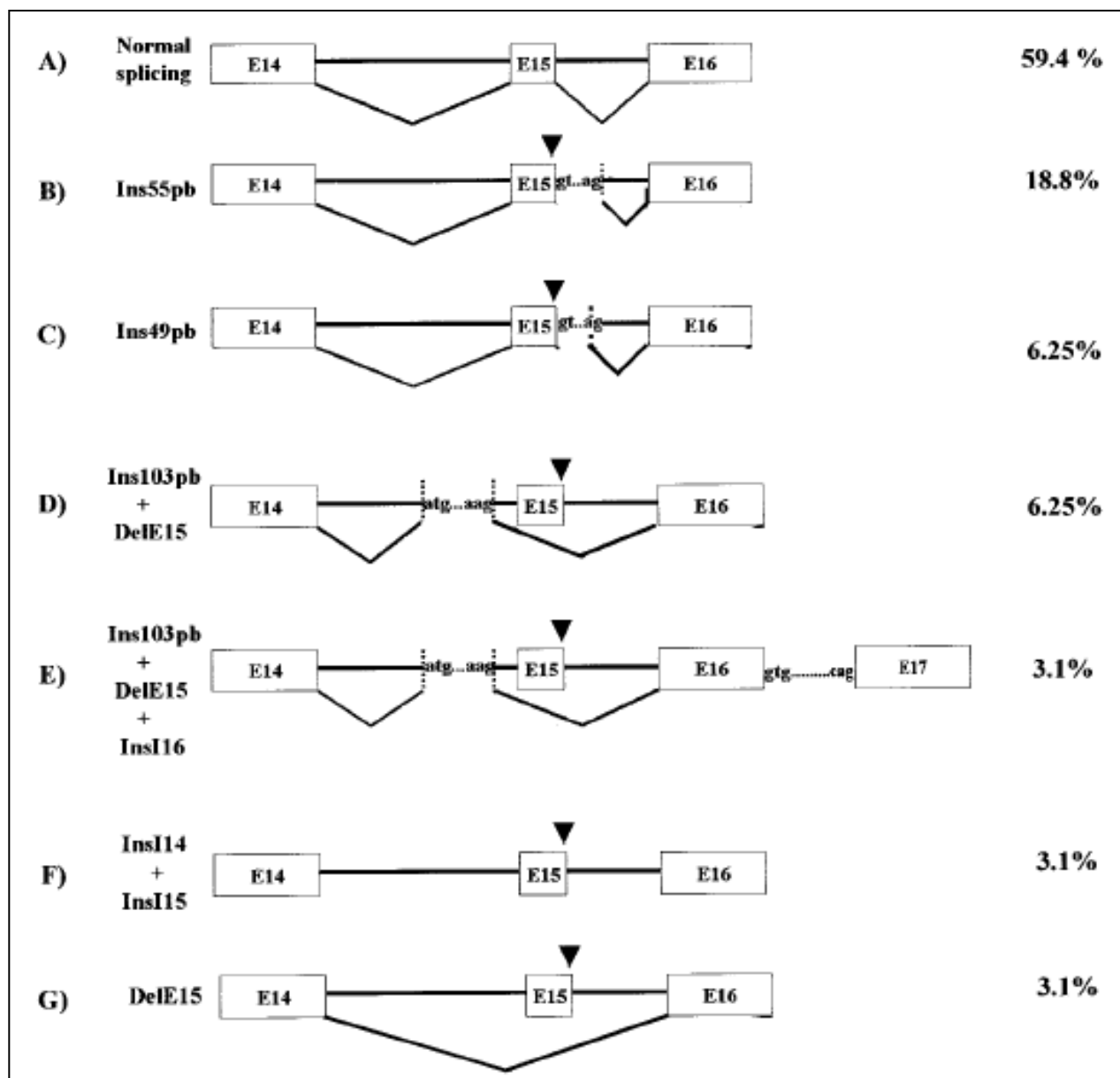
No PTC: p.W798R/p.W798R



Mutaciones “silentes” ???; efectos mRNA

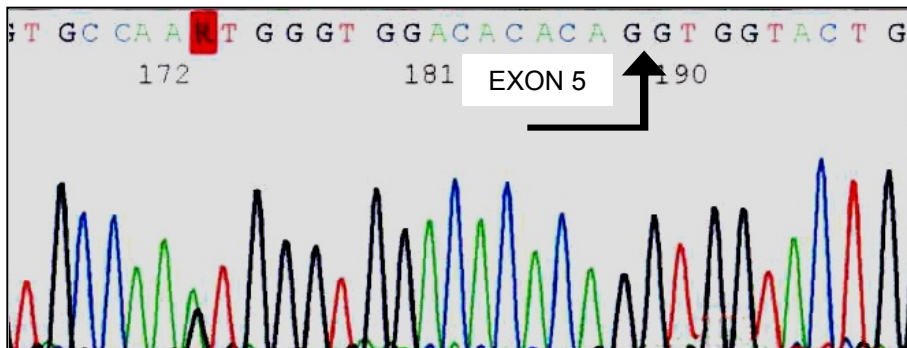
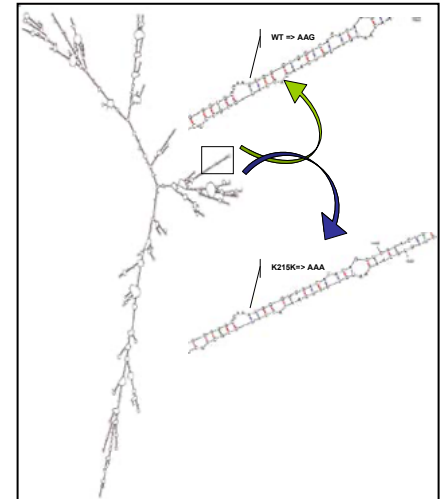
W798R/ K609K





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)

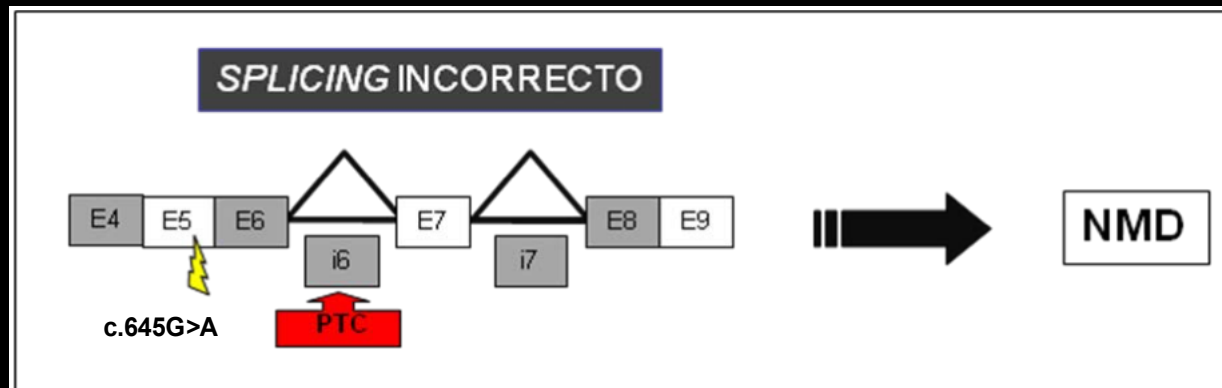
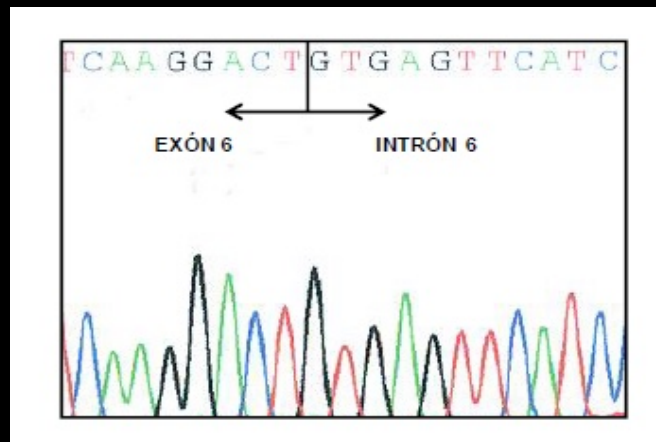


PATOGENICA:

- 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.



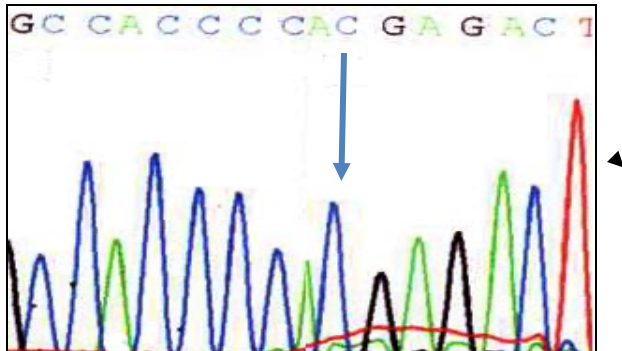
(p.R50X + p.V657_G726del)

ADNc MÚSCULO

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

HOMO

p.R50X WT

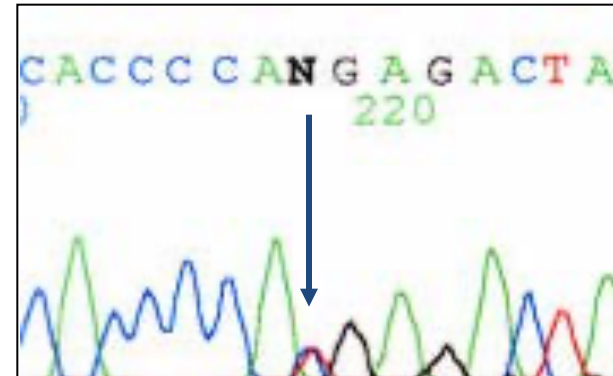


ADNc SANGRE

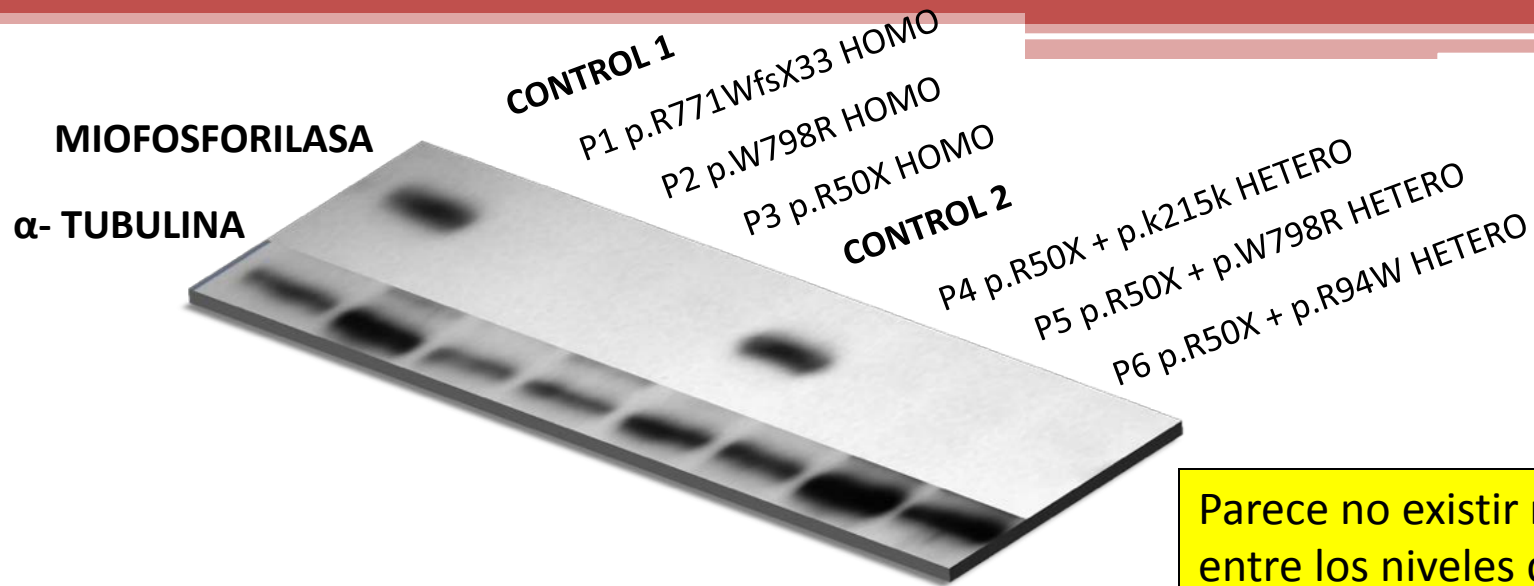
c.(1969+214)_(2177+369)del

HETEROCIGOSIS

p.R50X

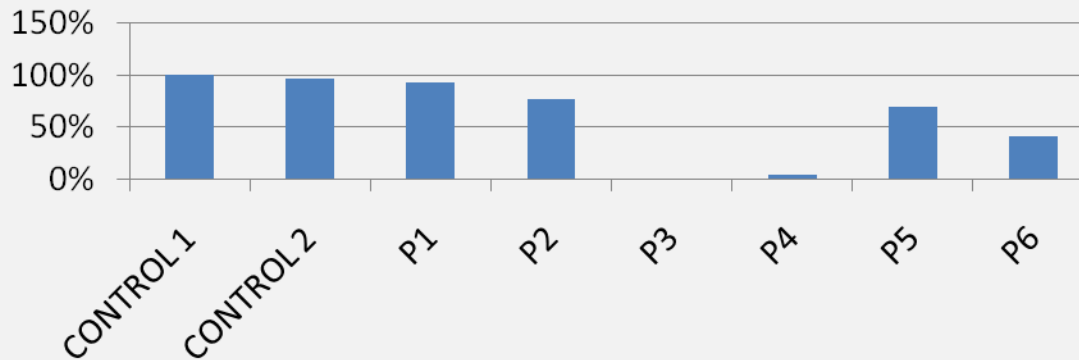


CARACTERIZACION DE LAS ALTERACIONES PROTEÍCAS EN LA ENFERMEDAD DE McARDLE



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

Niveles Transcritos gen *PYGM*



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

Análisis monodimensional

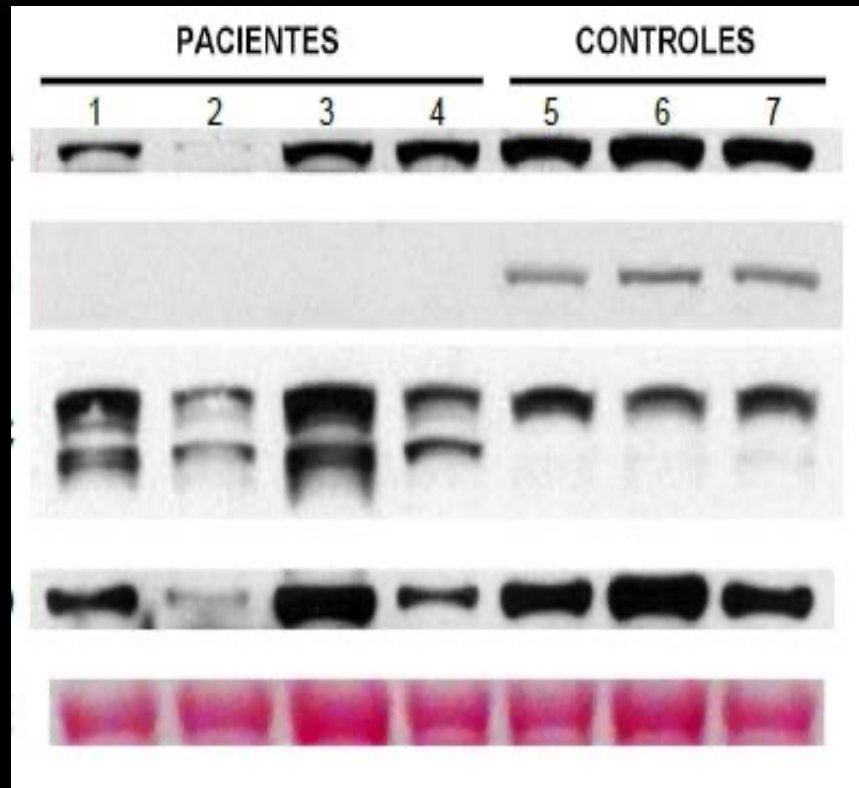
SERCA1
104 KD

GPM
97 KD

GS
84 KD

α -Tubulina
55 KD

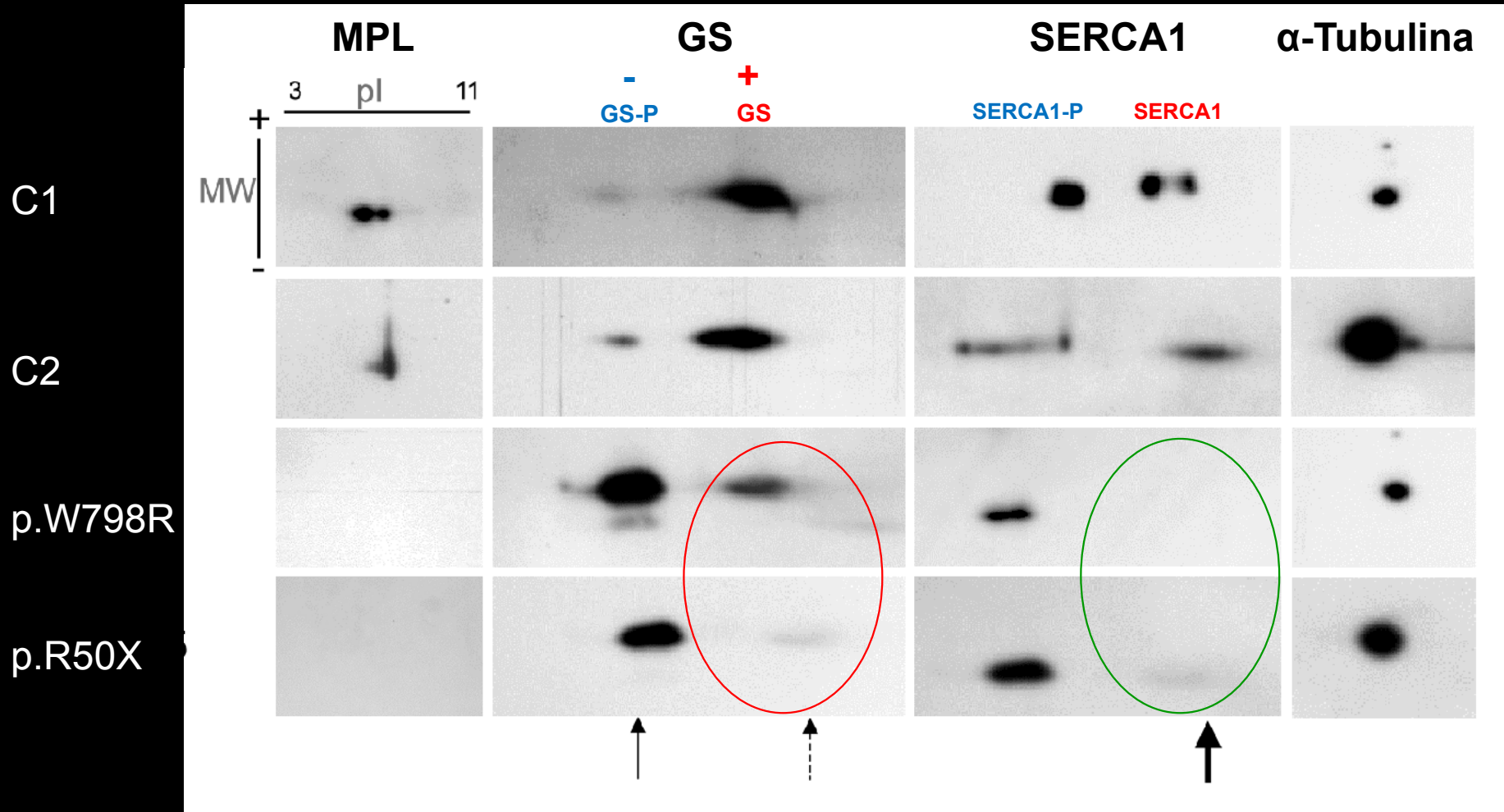
Miosina
217 KD



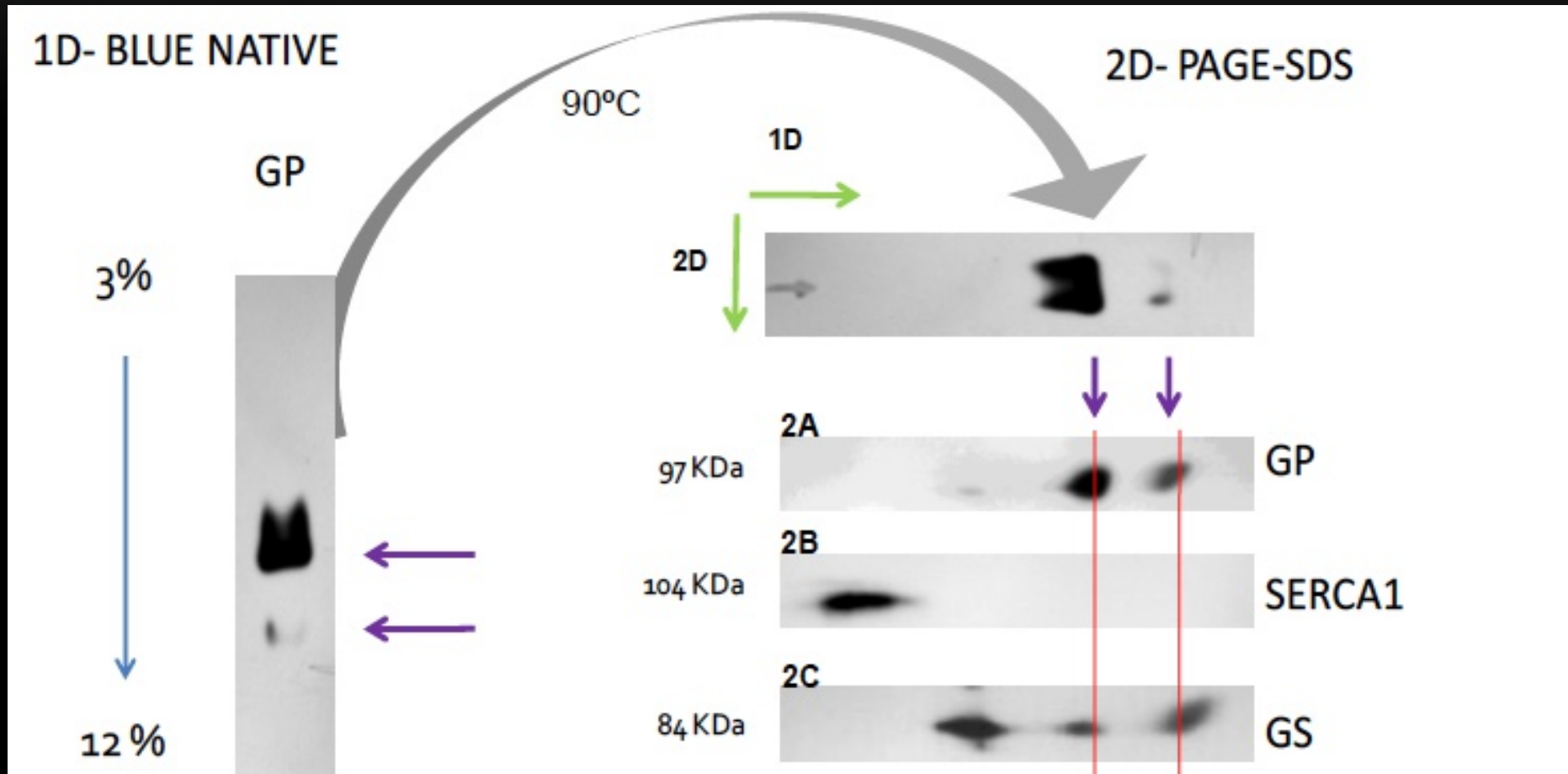
PACIENTES

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

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?



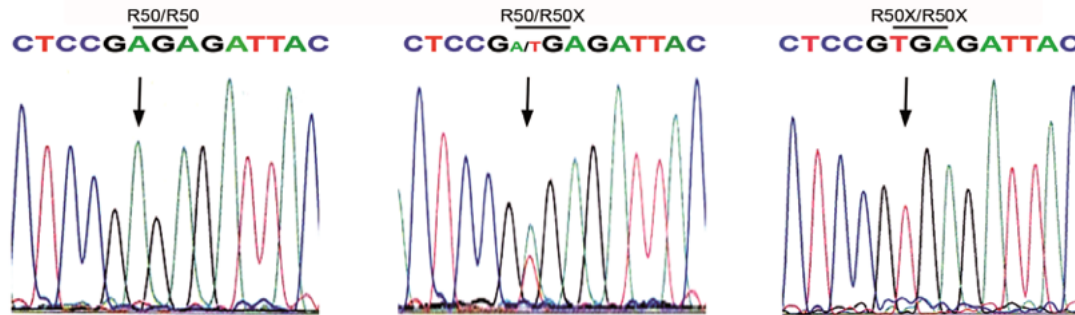
- The knock-in mouse model present the most frequent mutation in McArdle patients (R.50X).
- The knock-in introduction of the R.50X mutation introduces a premature termination codon (PTC) which potentially induces non-sense mediated decay (NMD). It allow us to study NMD processes and possible therapeutic 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

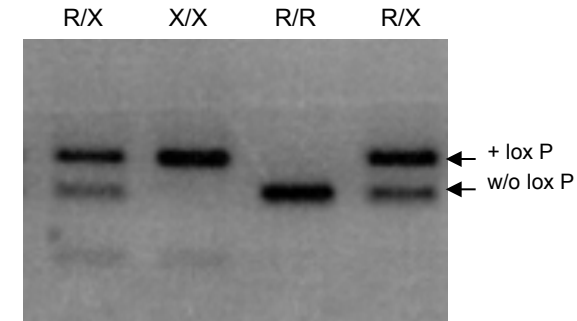


GENOTYPING MICE

by sequencing



by lox p analysis

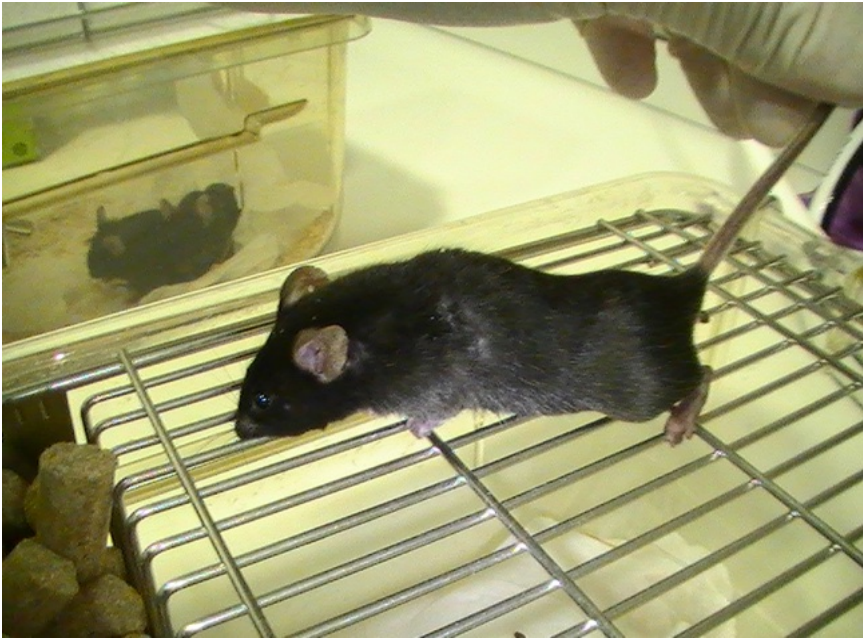


	Dimerization domain	p.R50	
SHEEP	MSRPLTDQEKRKQISVRGLAGVENVTELKKNFNRHLHFTLVKDRNVATERDYYFALAYTV		60
CATTLE	MSRPLTDQEKRKQISVRGLAGVENVTELKKNFNRHLHFTLVKDRNVATERDYYFALAYTV		60
MOUSE	MSRPLSDQDKRKQISVRGLAGVENVSELKKNFNRHLHFTLVKDRNVATERDYYFALAHTV		60
RABBIT	MSRPLSDQEKRKQISVRGLAGVENVTELKKNFNRHLHFTLVKDRNVATERDYYFALAHTV		60
HUMAN	MSRPLSDQEKRKQISVRGLAGVENVTELKKNFNRHLHFTLVKDRNVATERDYYFALAHTV		60
SALMON	MPKPLTDQEKRKQISVRGLAGVENVSDLKTNFNRHLHFTLVKDRNVATKRDYYFALANTV		60
	*.:**:**:*****:.*.*.***** **		

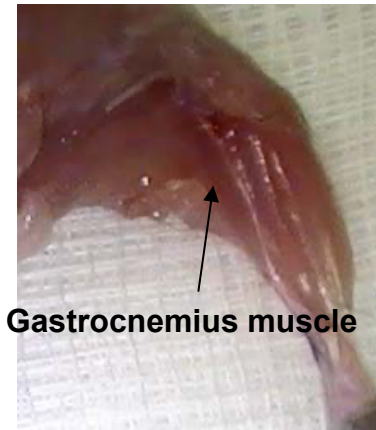
98% protein homology between human and mouse

82% protein homology between human and salmon

MUSCLE EXTRACTION



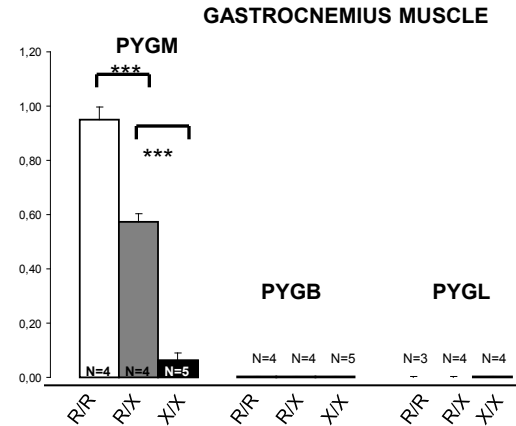
HOMOZYGOUS R50X MOUSE



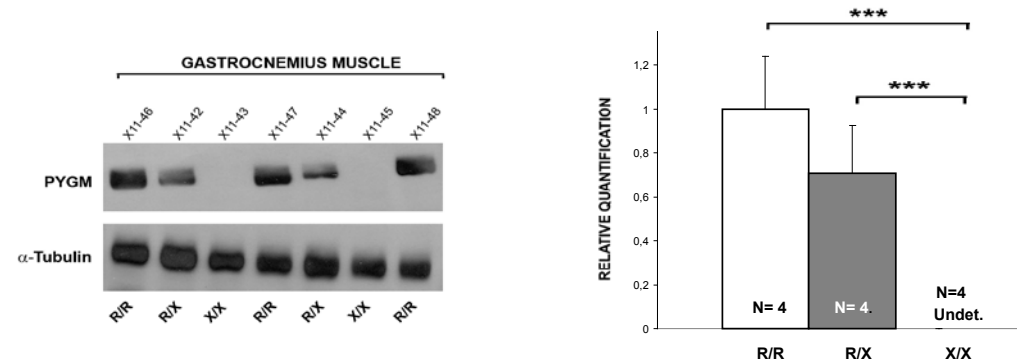
HOMOZYGOUS R50X MOUSE

MOLECULAR PHENOTYPE

- mRNA levels



- Protein levels



- GP activity levels

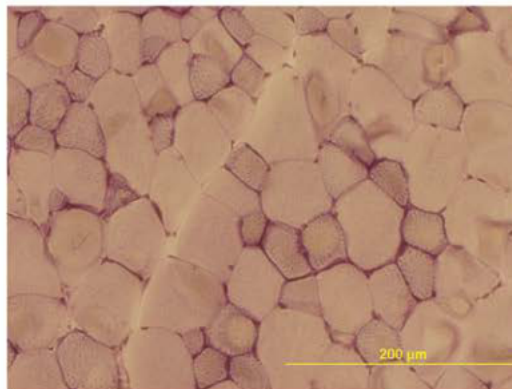
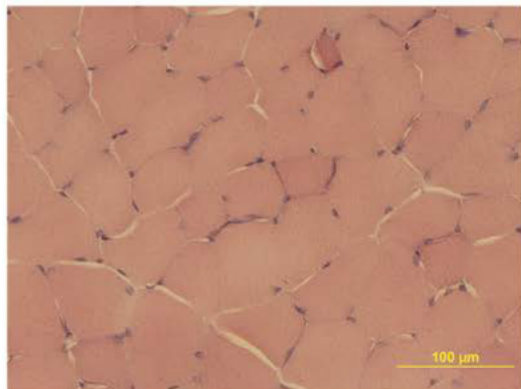


HE

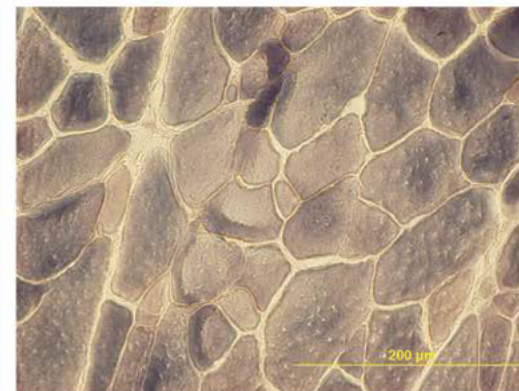
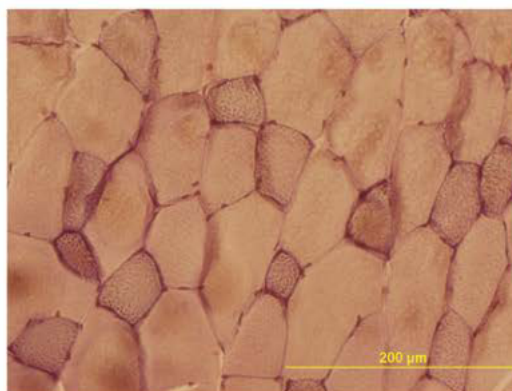
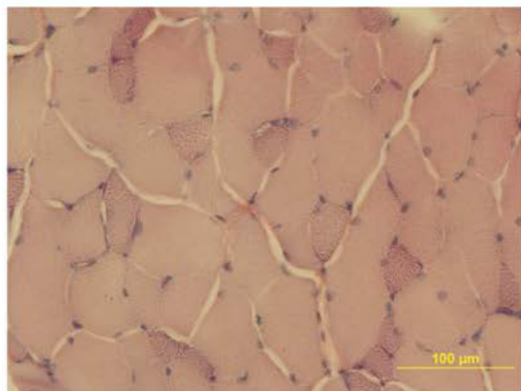
PAS

GP

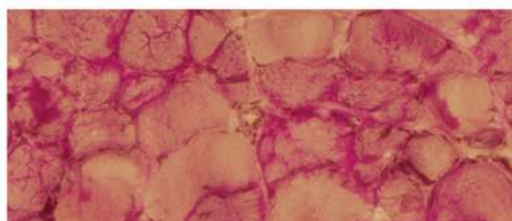
R/R



R/X



X/X



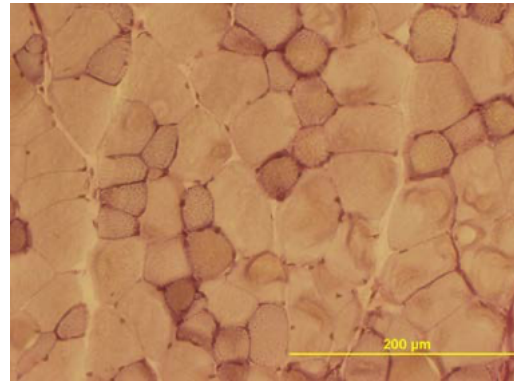
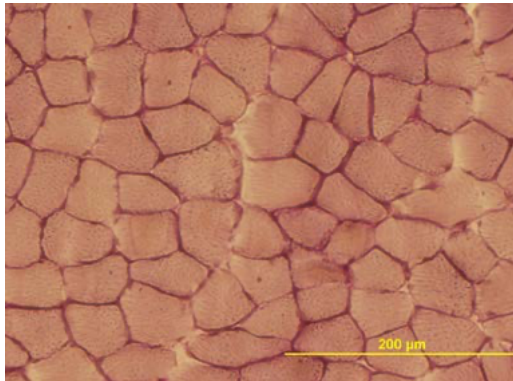
PAS STAINING IN DIFFERENT MUSCLES

SOLEUS

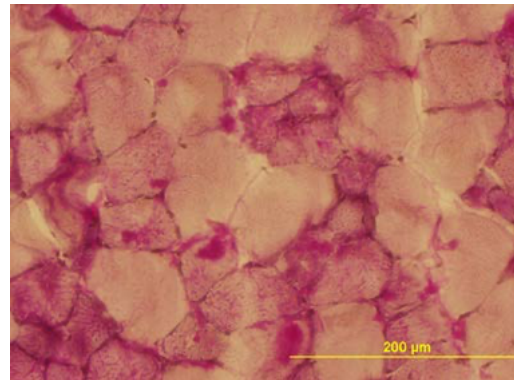
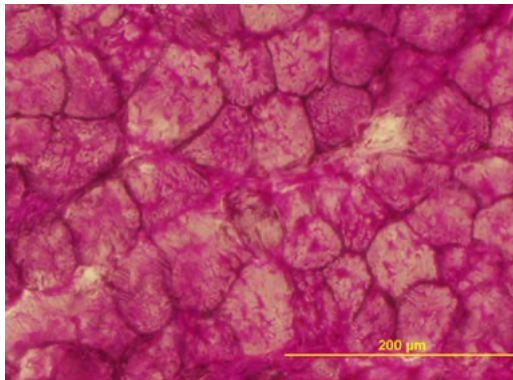
BICEPS

GASTROCNEMIUS

R/R



X/X

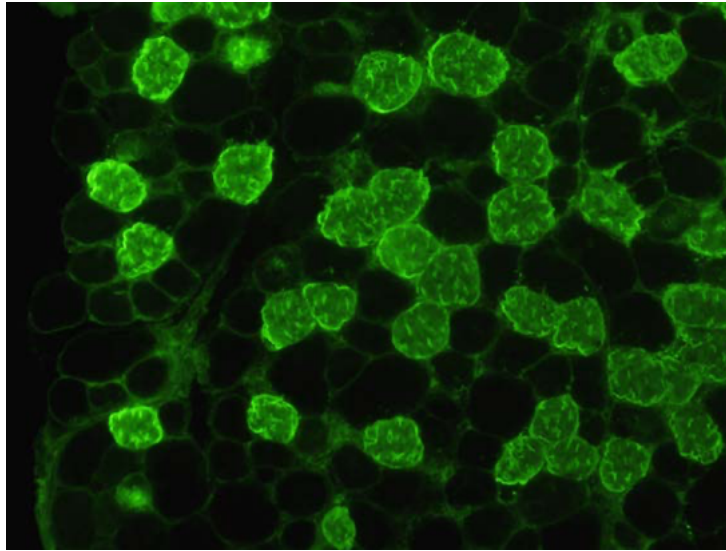


oxidative

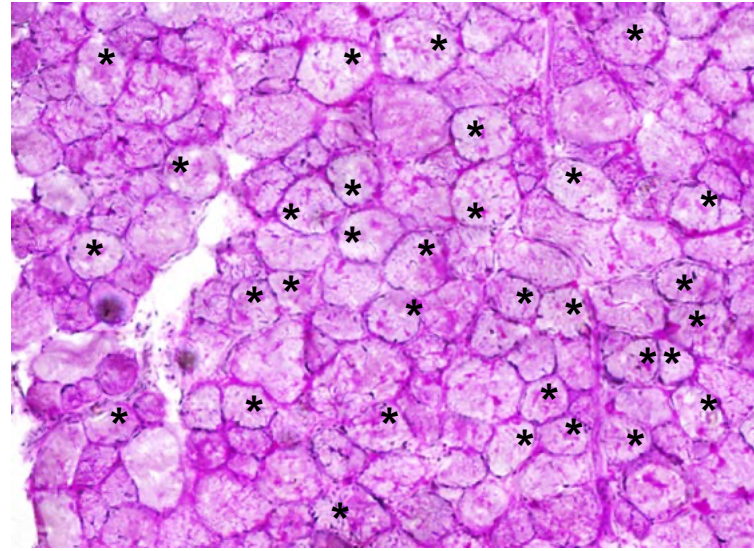
glycolytic

mixed

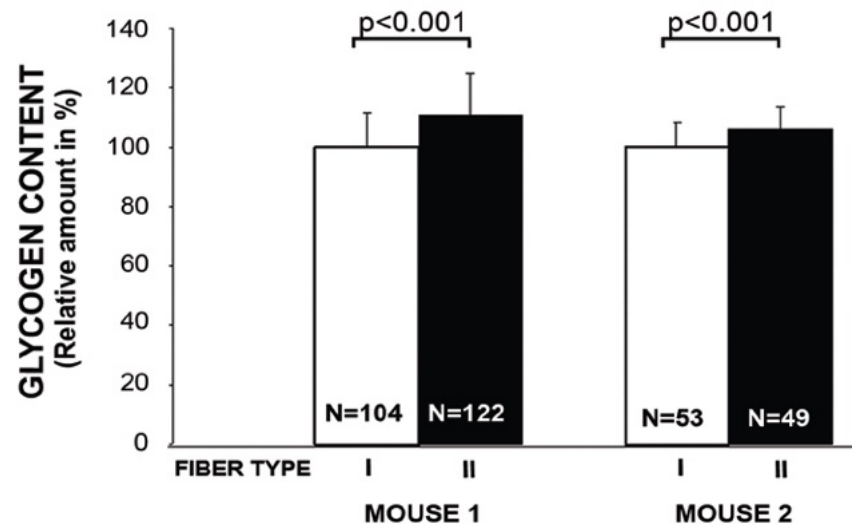
HIGHER GLYCOGEN ACCUMULATION IN TYPE II FIBERS



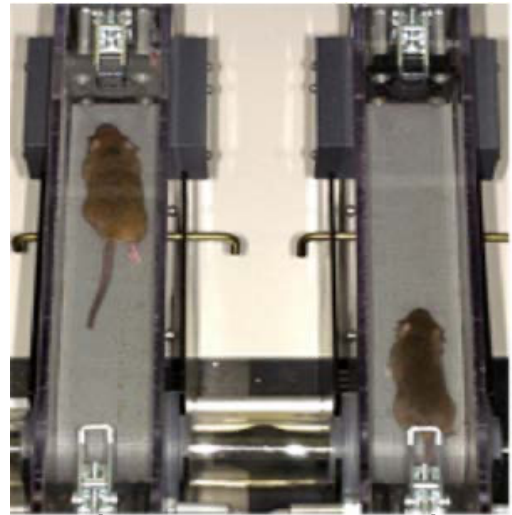
MHC type I



PAS-staining



TREADMILL EXPERIMENT



ELECTRIC SHOCK : 0.2 mA

EXPERIMENT DESIGN:

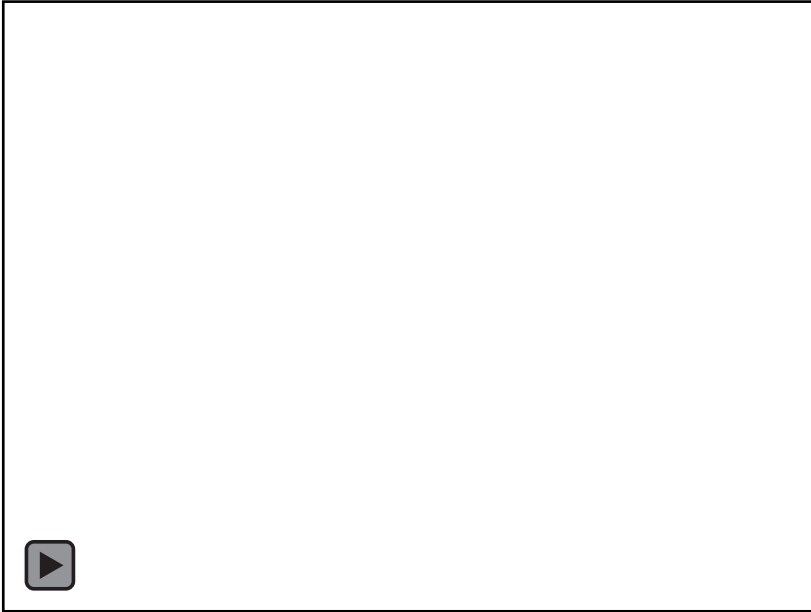
Inclination : 45°



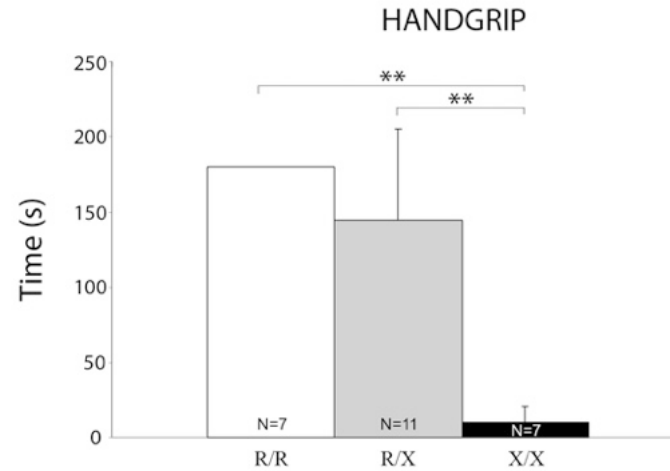
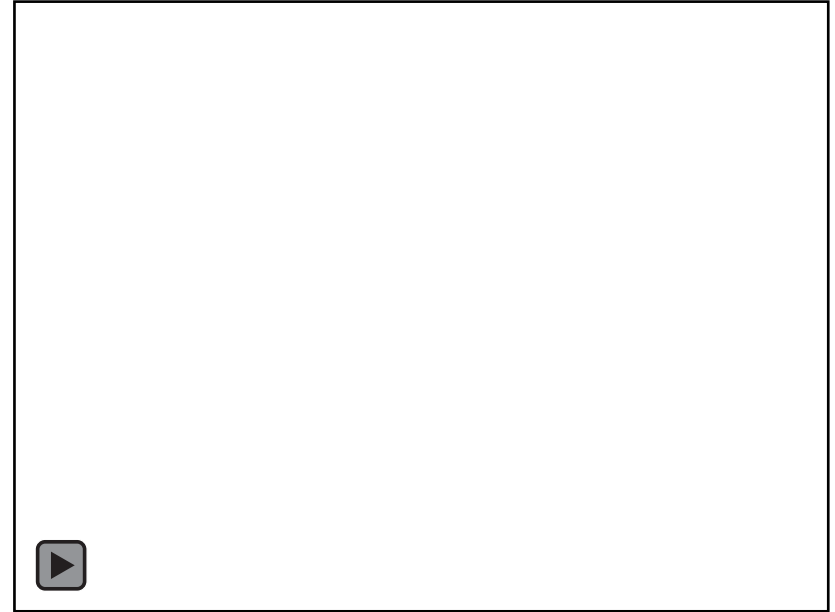
Stop: A) When more than 50% of time in the electric grid or B) more than 5 consecutive seconds in the electric grid

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.

