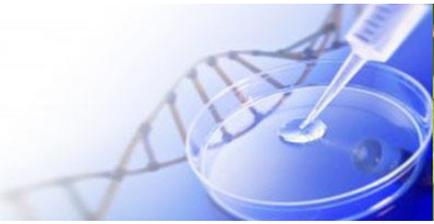




Distrofia Miotónica de Steinert: Nuevas estrategias terapéuticas

M.Carmen Álvarez
Valentia Biopharma



Distrofia Miotónica de tipo 1

Distrofia Miotónica de tipo 1 (DM1)

Cuadro clínico básico de la enfermedad

- ...la DM es, probablemente, la enfermedad humana más variable en sus síntomas...
- ...afecta de algún modo a virtualmente todos los sistemas del cuerpo...
- ...la DM puede presentarse en cualquier momento durante la vida del individuo...

Enfermedad rara

Prevalencia de 1/8.500 nacimientos vivos. Prevalencia mayor en algunas áreas como Quebec (Canadá)

Cuadro clínico básico de la enfermedad

Major Effects of Myotonic Dystrophy Type 1

www.myotonicdystrophy.com

Cognitive Function: Intellectual impairment, behavioral and psychological disorders, excessive daytime sleepiness

Vision: Cataracts, retinal damage

Endocrine System: Diabetes, low thyroid hormone levels

Respiratory System: Breathing difficulties, aspiration, sleep apnea, high risk pneumonia

Skin: Piliomatrixomas

Immune: Hypogammaglobulinemia

Reproductive System in Men: Low testosterone levels, erectile dysfunction, testicular failure and gonadal atrophy.

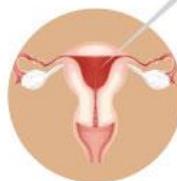
Bone: Anomalies

Cardiovascular System: Heart condition abnormalities, arrhythmias, cardiomyopathy

Gastrointestinal Tract: Swallowing issues, abdominal pain, irritable bowel syndrome, constipation/diarrhea, poor nutrition and weight loss, chronic infections

Muscle: Weakness, wasting (atrophy), myotonia, pain

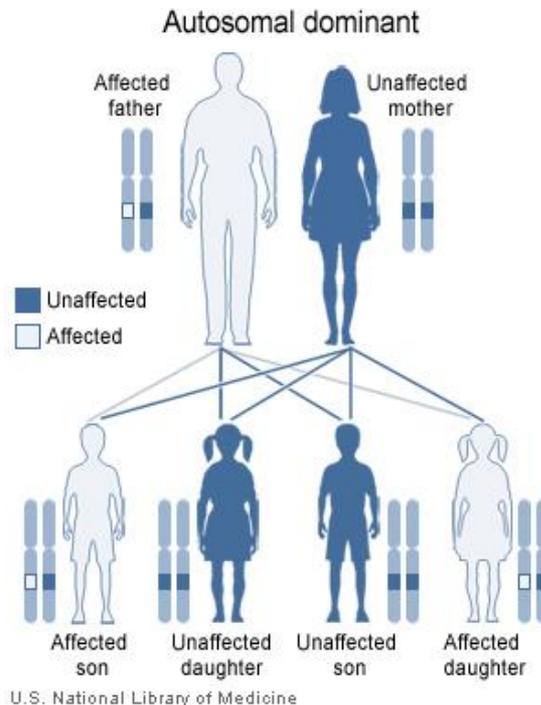
Reproductive System in Women: Weakened uterine muscle, pregnancy-related complications, and gynecological problems.



DM afecta a múltiples órganos y sistemas

Cuadro clínico básico de la enfermedad

- Congénita. Transmisión materna
- De inicio infantil
- De inicio adulto
- De inicio tardío o asintomático

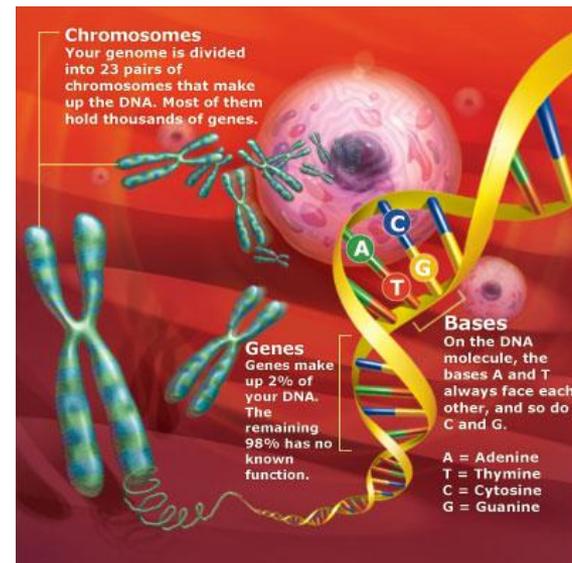


- Genética
- Hereditaria
- Autosómica
- Dominante



Base genética de la enfermedad

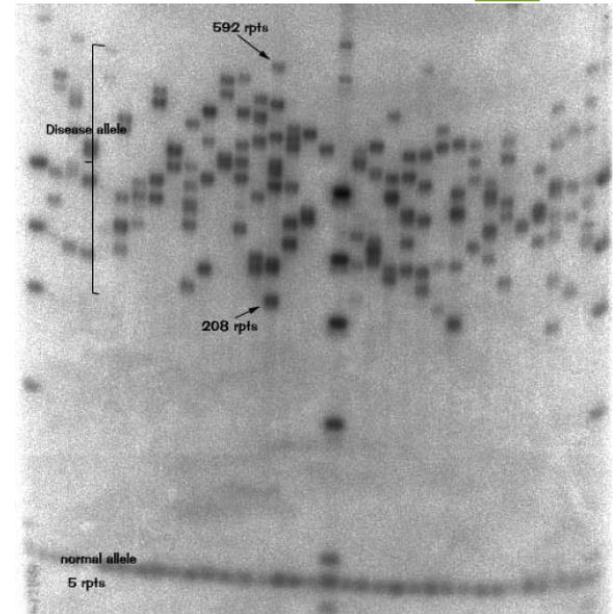
La mutación responsable de la DM1 se localiza en el gen DMPK, en el cromosoma 19



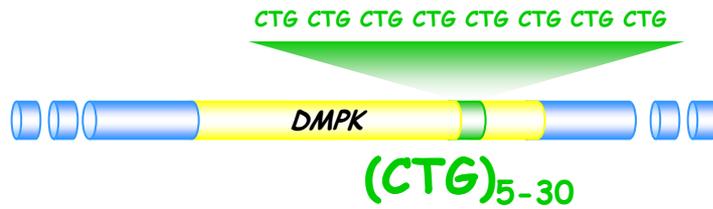
Base genética de la DM1: inestabilidad somática y germinal

El número de repeticiones CTG en el gen *DMPK* varía:

- Entre 5 y 37 repeticiones de padres a hijos sanos
- Aumenta mucho de padres afectados a hijos afectados
 - La enfermedad suele aparecer antes y ser más grave en generaciones sucesivas
- De una célula a otra en los pacientes
 - Probablemente contribuye a la variabilidad en los síntomas

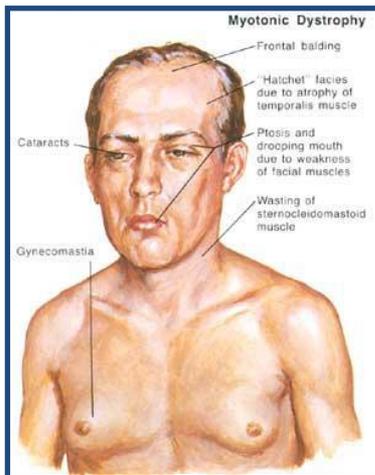


De los tripletes CTG a los síntomas



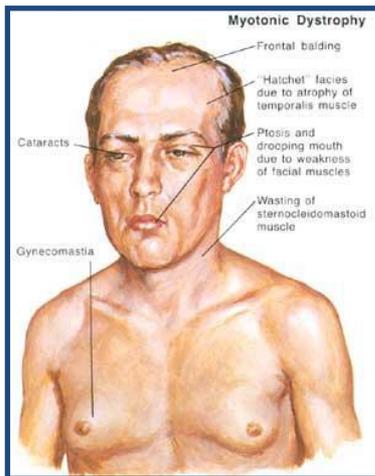
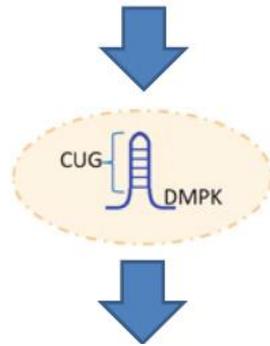
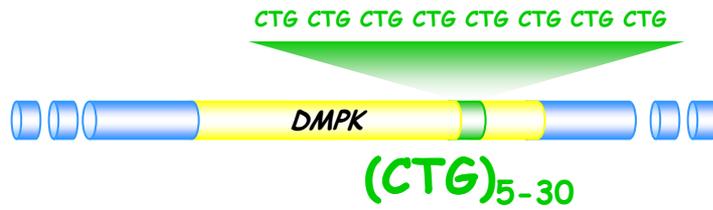
Expansión ADN

???



Síntomas

De los tripletes CTG a los síntomas

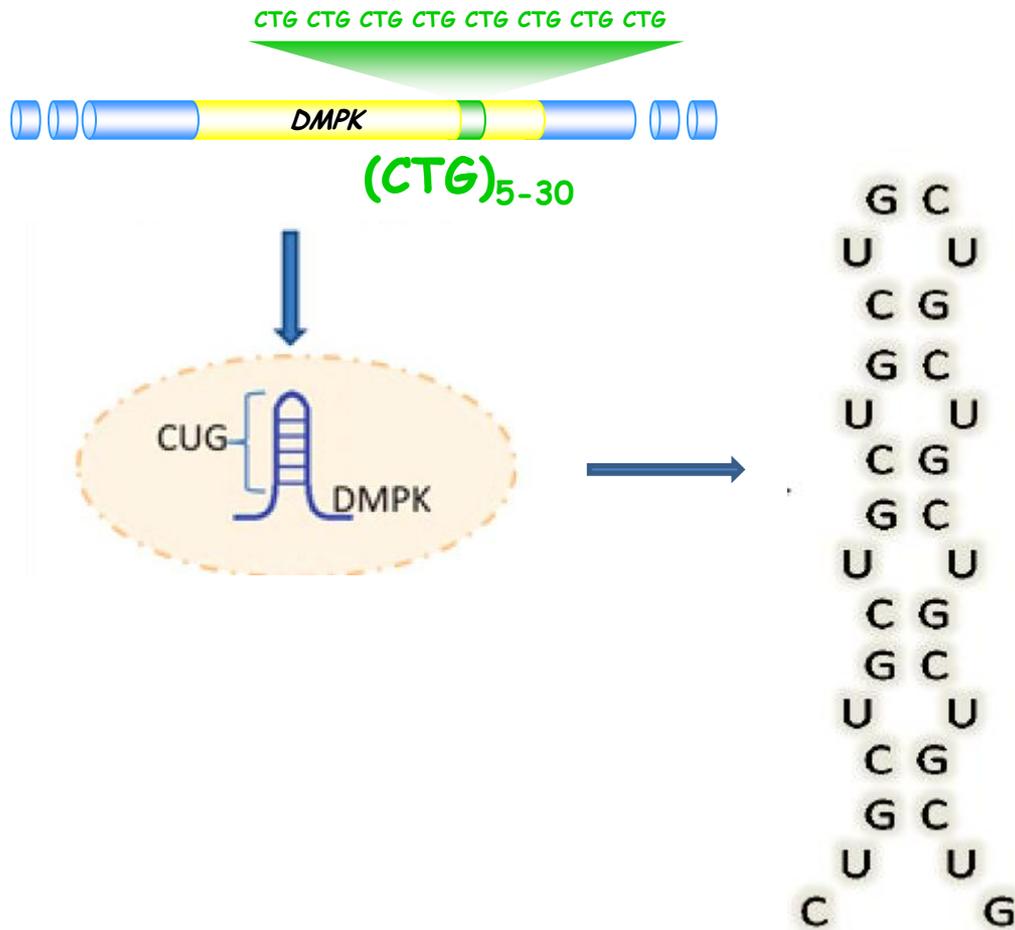


Expansión ADN

Ganancia de función del ARN
(ARN tóxico)

Síntomas

Mecanismo de patogénesis

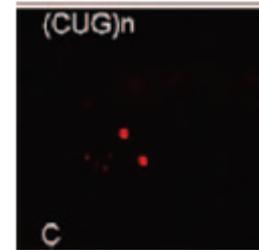
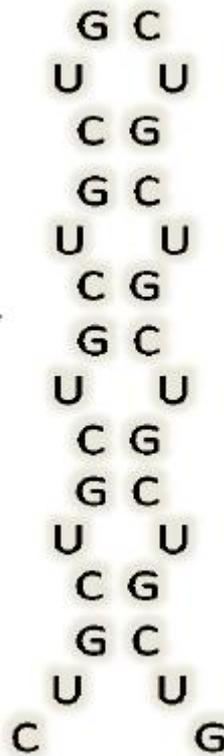
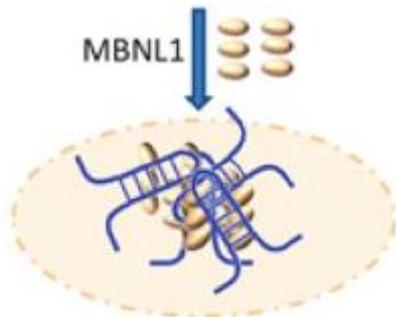
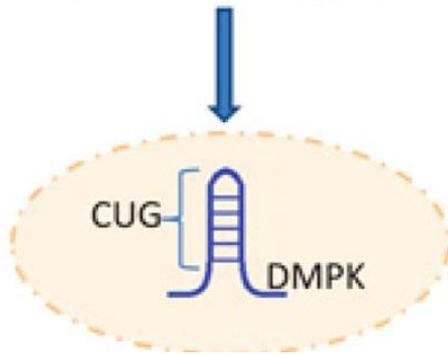


Formación de la horquilla

Mecanismo de patogénesis



(CTG)₅₋₃₀



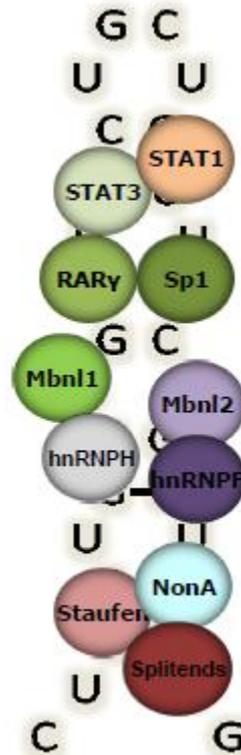
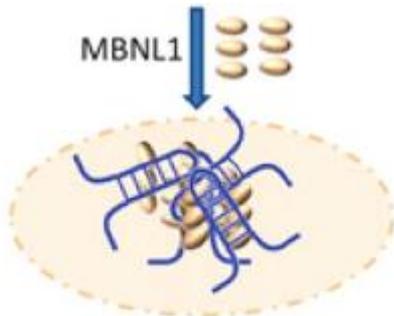
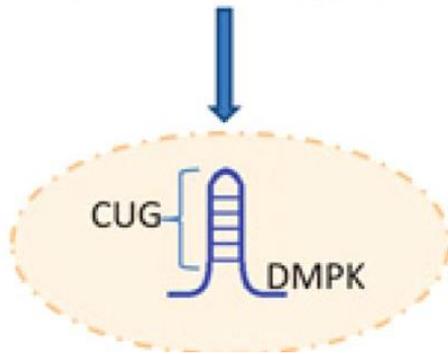
Mandoki et al., 2005 Circ. Res;97:1152-1155;

Formación de *foci*

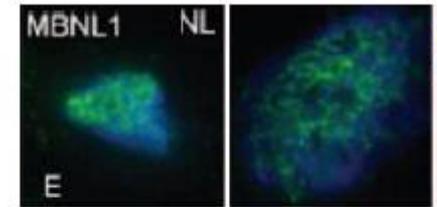
Mecanismo de patogénesis



(CTG)₅₋₃₀

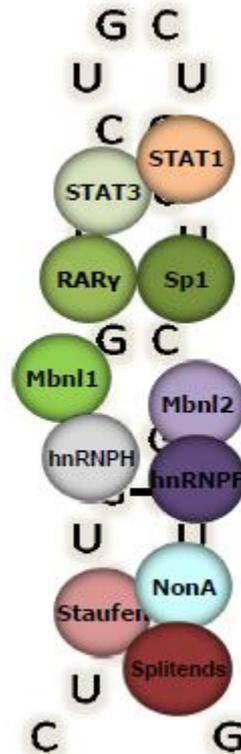
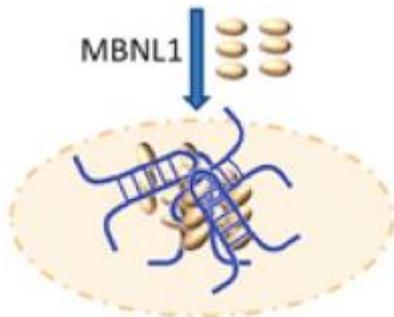
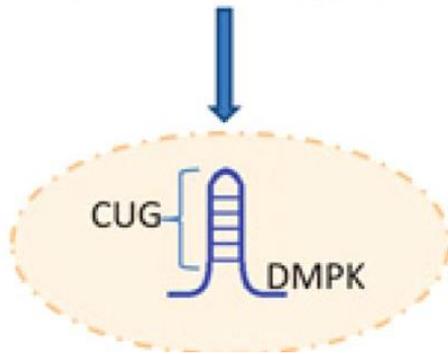
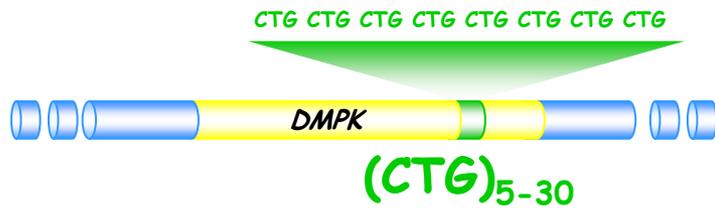


Secuestro de proteínas



Mandoki et al., 2005 Circ. Res;97;1152-1155;

Mecanismo de patogénesis

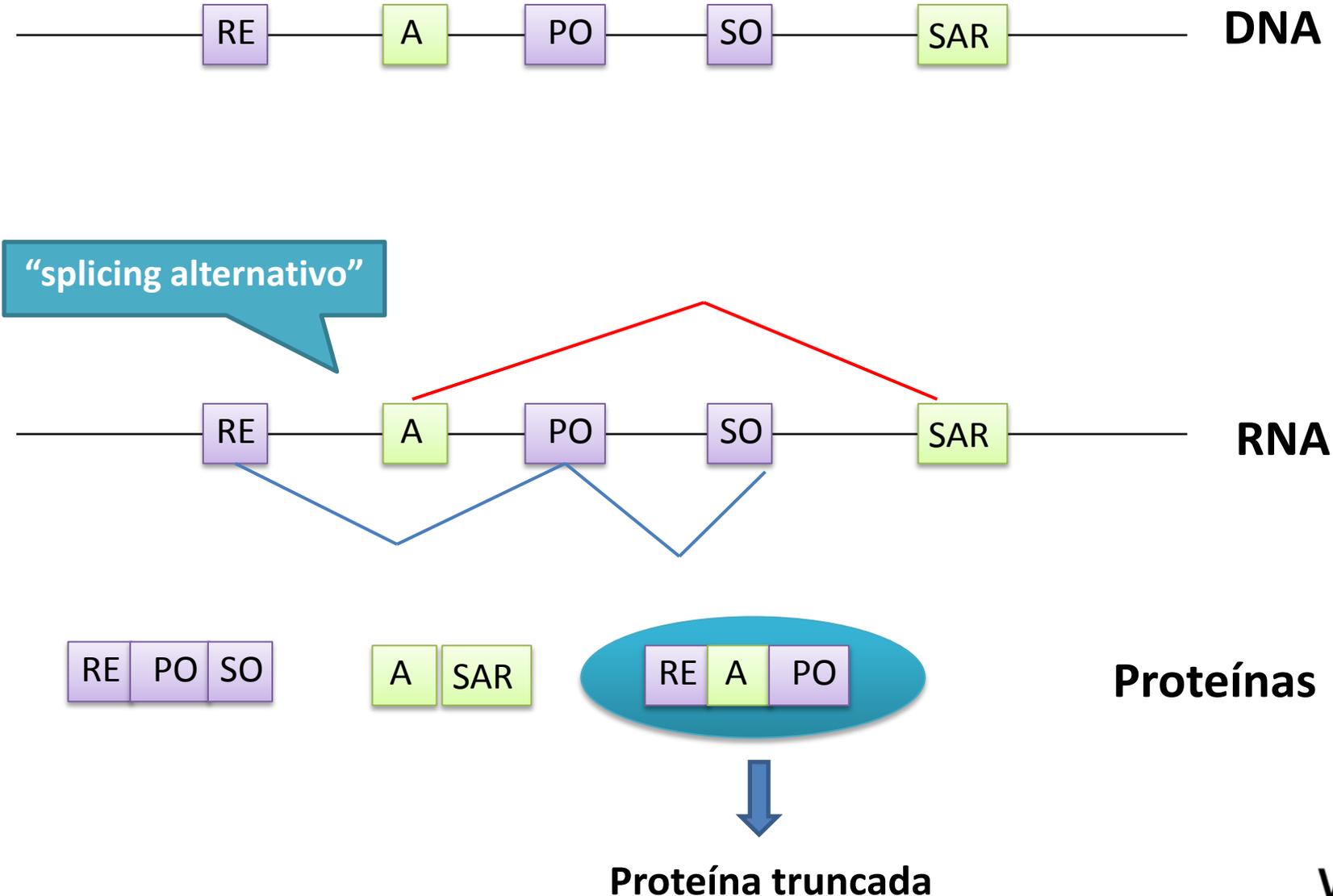


(varios genes)

- CIC1
- Tnnt2
- Tnnt3
- Serca1
- IR

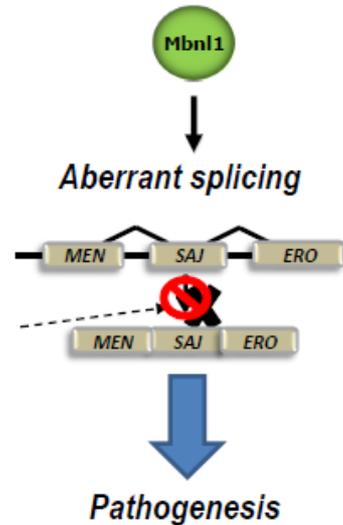
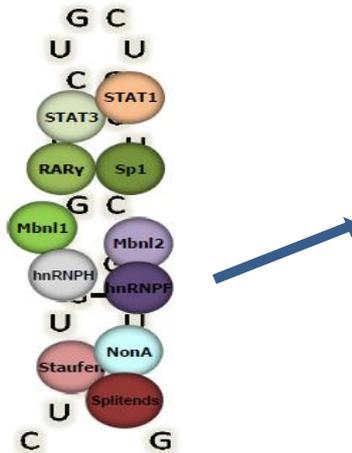
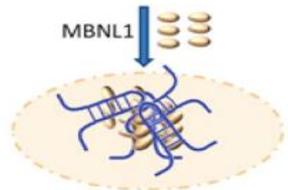
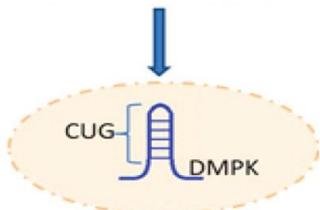
Secuestro de proteínas

Mecanismo de patogénesis: *splicing alternativo*



Mecanismo de patogénesis: *splicing alternativo*

A nivel molecular la DM es un problema en el “splicing alternativo” de muchos genes

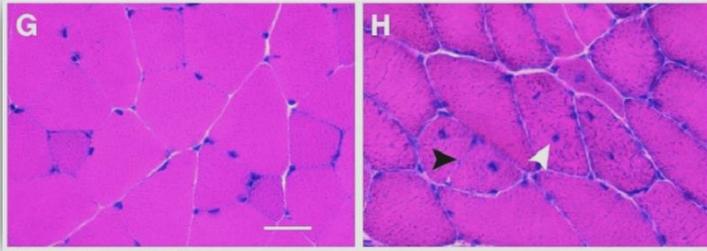


Aberrant splicing	Insulin Receptor	Chloride Channel CLCN1	Cardiac Troponin T	RYR1 MTMR1	NMDAR1 Tau APP
	↓	↓	↓	↓ Bin-1	↓
Disease features	Insulin Resistance	Myotonia	?cardiac abnormalities	?Muscle weakness and wasting	CNS effects

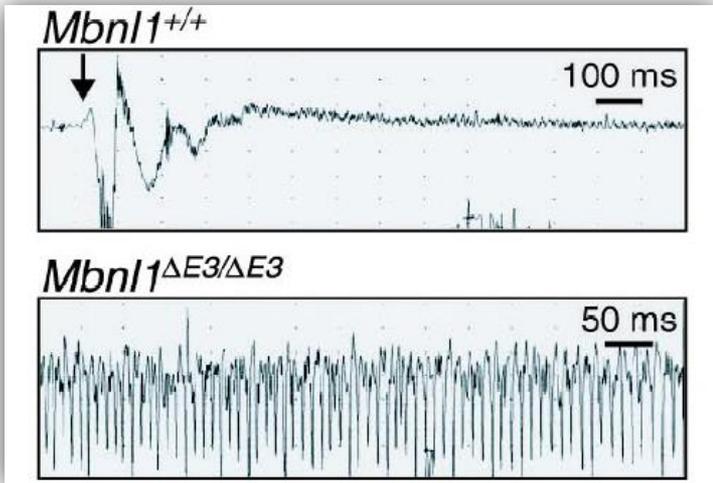
La importancia de *Mbnl1* en DM1

wt

Mbnl1^{-/-}



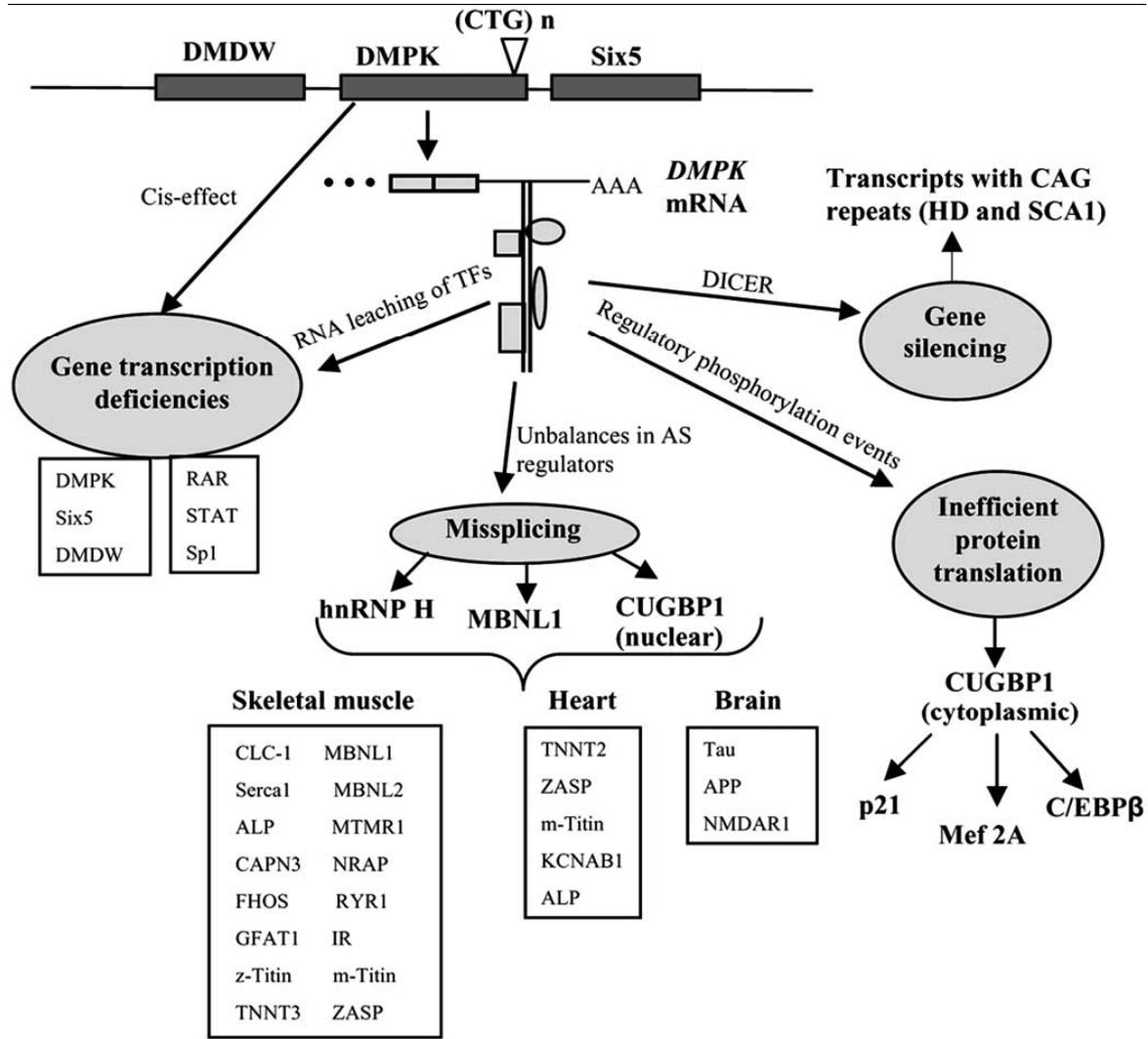
	<i>Mbnl1</i> ^{-/-}	Pacientes DM1
<i>Clcn1</i>	+	+
<i>Sercal</i>	+	+
<i>Tnnt3</i>	+	+
ZASP	+	+
<i>z-Ttn</i>	+	+
<i>z-Ttn</i>	+	+
<i>m-Ttn</i>	+	+
<i>Capn3</i>	+	+
<i>Alp</i>	+	+
<i>Fhos</i>	+	+
<i>Gfat1</i>	+	+
<i>Mbnl1</i>	+	+
<i>Mbnl2</i>	+	+



Kanadia et al., 2003

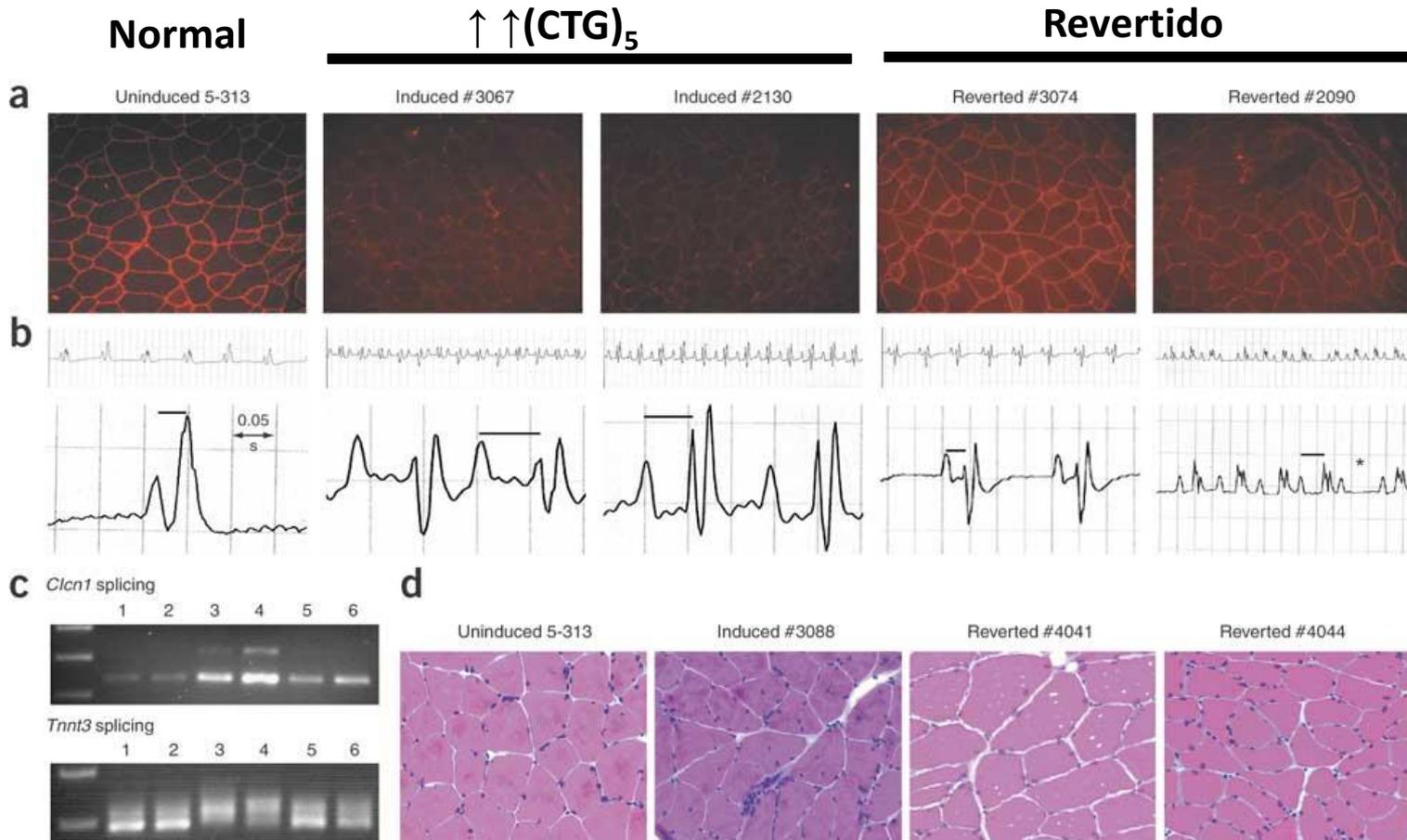
Generación de un ratón deficiente en *Mbnl1*

La DM1 es una enfermedad muy compleja



La DM1 es reversible en ratones

Podemos ser optimistas: la distrofia miotónica es reversible en ratones

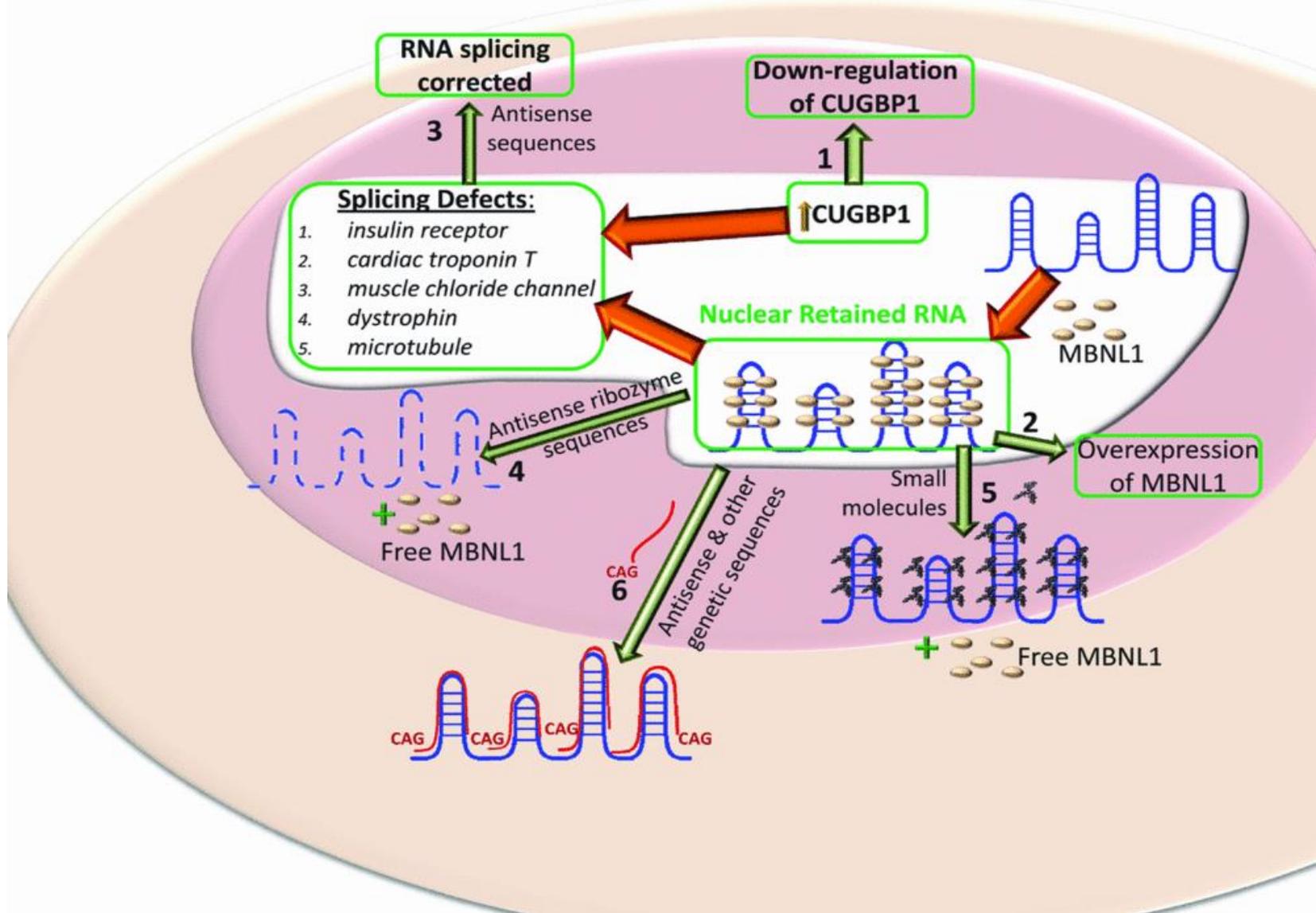


Avances en la investigación en DM1

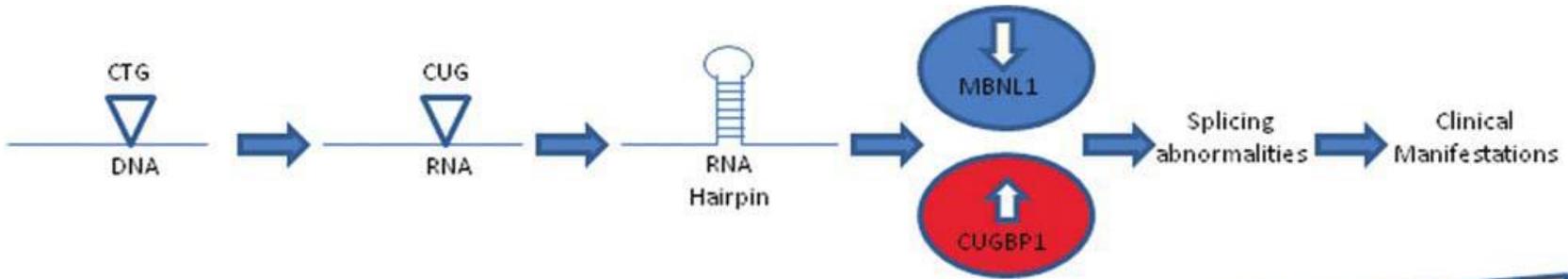
Por el momento, el tratamiento actual se basa en paliar cada uno de los síntomas.....

.....Sin embargo se ha avanzado mucho en la comprensión de la enfermedad y se está trabajando sobre diferentes estrategias terapéuticas, algunas de ellas con resultados prometedores en las fases preclínicas.....

Diferentes estrategias.....



Diferentes estrategias.....



Número de procesos celulares implicados

Impacto sobre la enfermedad

Facilidad de la invención

RNAs antisentido

Reversal of RNA Dominance by Displacement of Protein Sequestered on Triplet Repeat RNA

Thurman M. Wheeler,¹ Krzysztof Sobczak,¹ John D. Lueck,² Robert J. Osborne,¹
Xiaoyan Lin,¹ Robert T. Dirksen,² Charles A. Thornton^{1*}

Genomic expansions of simple tandem repeats can give rise to toxic RNAs that contain expanded repeats. In myotonic dystrophy, the expression of expanded CUG repeats (CUG^{exp}) causes abnormal regulation of alternative splicing and neuromuscular dysfunction. We used a transgenic mouse model to show that derangements of myotonic dystrophy are reversed by a morpholino antisense oligonucleotide, CAG25, that binds to CUG^{exp} RNA and blocks its interaction with muscleblind-like 1 (MBNL1), a CUG^{exp}-binding protein. CAG25 disperses nuclear foci of CUG^{exp} RNA and reduces the overall burden of this toxic RNA. As MBNL1 is released from sequestration, the defect of alternative splicing regulation is corrected, thereby restoring ion channel function. These findings suggest an alternative use of antisense methods, to inhibit deleterious interactions of proteins with pathogenic RNAs.

Myotonic dystrophy type 1 (DM1) is representative of a group of dominantly inherited disorders in which expression of a toxic RNA leads to neuromuscular degeneration (1-5). A feature common to these pathogenic RNAs is the presence of an expanded

INVESTIGADOR PRINCIPAL:
Charles Thornton
(Universidad de Rochester)

336 17 JULY 2009 VOL 325 SCIENCE www.sciencemag.org

MOLÉCULA: RNA morpholino antisentido (CAG)₂₅

DIANA: la repetición CUG en las moléculas de RNA

MECANISMO DE ACCIÓN: Bloquea el secuestro de MBNL1. Los resultados muestran disminución de la cantidad de RNA tóxico que desencadena la reducción de fenotipos de la enfermedad (foci, splicing y miotonía). Resultados **terapéuticos observables tras 6 meses de la administración** (sin publicar).

MODELOS UTILIZADOS: ratón HSA^{LR} (ruta intramuscular).

DESARROLLO: Mejorando el antisentido para que funcione a nivel sistémico

RNAs antisentido

LETTER

doi:10.1038/nature21362

Targeting nuclear RNA for *in vivo* correction of myotonic dystrophy

Thurman M. Wheeler^{1,2}, Andrew J. Leger³, Sanjay K. Pandey⁴, A. Robert MacLeod⁴, Masayuki Nakamori^{1,2}, Seng H. Cheng⁵, Bruce M. Wentworth¹, C. Frank Bennett⁶ & Charles A. Thornton^{1,2}

Antisense oligonucleotides (ASOs) hold promise for gene-specific knockdown in diseases that involve RNA or protein gain-of-function effects. In the hereditary degenerative disease myotonic dystrophy type 1 (DM1), transcripts from the mutant allele contain an expanded CUG repeat^{1,2} and are retained in the nucleus^{3,4}. The mutant RNA exerts a toxic gain-of-function effect⁵, making it an appropriate target for therapeutic ASOs. However, despite improvements in ASO chemistry and design, systemic use of ASOs is limited because uptake in many tissues, including skeletal and cardiac muscle, is not sufficient to silence target messenger RNAs^{6,7}. Here we show that nuclear-retained transcripts containing expanded CUG (CUG⁹⁰) repeats are unusually sensitive to antisense silencing. In a transgenic mouse model of DM1, systemic administration of ASOs caused a rapid knockdown of CUG⁹⁰ RNA in skeletal muscle, correcting the physiological, histopathological and transcriptional features of the disease. The effect was sustained for up to 1 year after treatment was discontinued. Systemically administered ASOs were also effective for muscle knockdown of *Malat1*, a long non-coding RNA (lncRNA) that is retained in the nucleus⁸. These results provide a general strategy to correct RNA gain-of-function effects and to modulate the expression of expanded repeats, lncRNAs and other transcripts with prolonged nuclear residence.

with oligomers⁹. As an alternative, RNase H-active ASOs could produce widespread correction, provided that uptake of circulating ASOs was sufficient to induce target cleavage.

We identified ASOs showing a strong knockdown of hACTA1 in tissue culture, good tolerability when systemically administered in wild-type mice, and activity against hACTA1-CUG⁹⁰ transcripts *in vivo* when electroporated into muscle (Supplementary Figs 1–3). The ASOs had 2'-O-methoxyethyl (MOE) modifications at both ends to maximize biostability, and a central gap of 10 unmodified nucleotides to support RNase H activity (MOE gapmers; Supplementary Table 1). We tested three of the ASOs in HES⁹⁰ transgenic mice by subcutaneous injection of 25 mg kg⁻¹ twice weekly (Fig. 1a). After 4 weeks of administration (8 injections), ASO 445236 reduced the level of CUG⁹⁰ RNA in hindlimb muscles by more than 80% (Fig. 1b). Another ASO targeting the 3' UTR, downstream of the repeat tract, also showed strong CUG⁹⁰ reduction, whereas an ASO targeting the 5' UTR, or three oligonucleotides against other targets, had no effect (Fig. 1b, c).

RNase H cleavage of mRNA is usually followed by rapid decay of cleavage fragments. However, stable cleavage fragments are observed occasionally¹⁰, and the CUG⁹⁰ tract forms extensive hairpins¹¹ and ribonucleoprotein complexes¹² that could inhibit exonuclease activity. The failure of antisense targeting in the 5' UTR also raised the

INVESTIGADOR PRINCIPAL:

Charles Thornton

(Universidad de Rochester)

EMPRESA: Genzyme, Isis Pharmaceuticals

MOLÉCULA: RNA-gapmer antisentido (29-O-methoxyethyl (MOE) modifications)

DIANA: regiones de RNA flanqueantes a la región expandida CUG

MECANISMO DE ACCIÓN: Activación de su degradación por RNasa H (enzima que degrada doble cadena de RNA). Los resultados muestran disminución de la cantidad de RNA tóxico que desencadena la reducción de fenotipos de la enfermedad (foci, splicing y miotonía). Resultados **terapéuticos observables tras un año de la administración.**

MODELOS UTILIZADOS: ratón HSA^{LR} (efecto sistémico por inyección subcutánea).

DESARROLLO: Genzyme apartado del proyecto. Acuerdo entre Isis y Biogen.

El futuro de los RNA antisentido

- Mejorar el mecanismo de administración: Utilización de vectores adenovirales (poca experiencia de su aplicación en humanos)
- Mejorar la biodisponibilidad: Conseguir que lleguen al cerebro
- Mejorar la vía de administración: Por el momento no se podría tener una administración oral
- Tecnología en constante evolución: Varias Universidades y empresas están trabajando en ello

Previsto un ensayo clínico durante el 2013 (Biogen+ Universidad de Rochester)

Small molecules

Pentamidine reverses the splicing defects associated with myotonic dystrophy

M. Bryan Warf¹, Masayuki Nakamori¹, Catherine M. Matthys², Charles A. Thornton², and J. Andrew Berglund^{1,2}

¹Department of Chemistry and Institute of Molecular Biology, University of Oregon, Eugene, OR 97403; and ²Department of Neurology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Edited by Jennifer A. Doudna, University of California, Berkeley, CA, and approved September 9, 2009 (received for review March 25, 2009)

Myotonic dystrophy (DM) is a genetic disorder caused by the expansion (as RNA) of expanded CUG or CCG repeats. The alternative splicing factor MBNL1 is sequestered to the expanded RNA repeats, resulting in missplicing of a subset of pre-mRNAs linked to symptoms found in DM patients. Current data suggest that if MBNL1 is released from sequestration, disease symptoms may be alleviated. We identified the small molecules pentamidine and neomycin B as compounds that disrupt MBNL1 binding to CUG repeats *in vitro*. We show in cell culture that pentamidine was able to reverse the missplicing of 2 pre-mRNAs affected in DM, whereas neomycin B had no effect. Pentamidine also significantly reduced the formation of ribonuclear foci in tissue culture cells, releasing MBNL1 from the foci in the treated cells. Furthermore, pentamidine partially rescued splicing defects of 2 pre-mRNAs in mice expressing expanded CUG repeats.

Results

Identification of Small Molecules That Disrupt an MBNL1-CUG Repeat Complex *In Vitro*. Twenty-six small molecules that are known to bind structured nucleic acid (Table S1) were screened to identify compounds that would disrupt a protein-RNA complex using a previously characterized MBNL1-CUG repeat gel shift assay (Fig. 1A) (3). The CUG RNA (CUG₄) used in this screen contains 4 repeats, which were stabilized into a stem-loop structure by using the ultrastable UUCG loop (Fig. 1A). The molecule that most effectively disrupted the complex was pentamidine, which competed with MBNL1 and disrupted the protein-RNA complex with an IC₅₀ of $58 \pm 5 \mu\text{M}$ (Fig. 1B-D). Neomycin B was the next best molecule with an IC₅₀ of $280 \pm 40 \mu\text{M}$ (Fig. 1B-D). The small molecules ethidium bromide and thiazole orange also disrupted the MBNL1-

SEE ON



MOLÉCULA: pentamidina

DIANA: la repetición CUG en las moléculas de RNA

MECANISMO DE ACCIÓN: Bloquea la interacción con proteínas como MBNL1.

DESARROLLO: En el último congreso de DM1 (2012) presentaron la heptamidina (modificación)

MOLÉCULA: bis-benzimidazole y modificaciones

DIANA: la repetición CUG en las moléculas de RNA

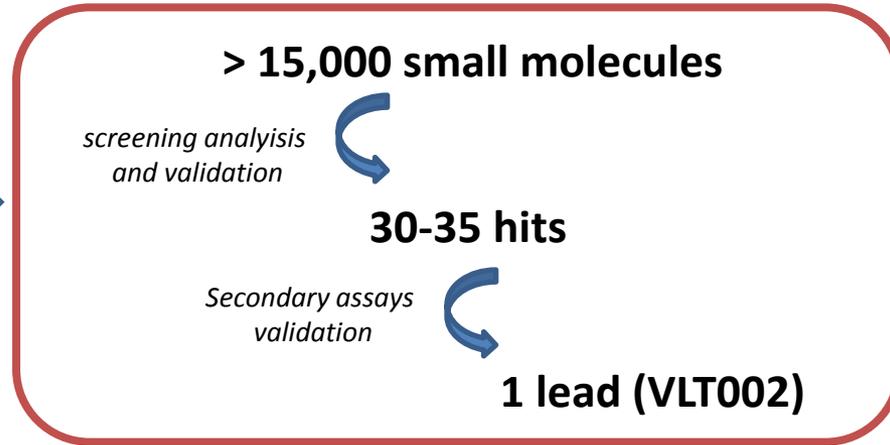
MECANISMO DE ACCIÓN: Bloquea la interacción con proteínas como MBNL1.

DESARROLLO: empresa SMaRT therapeutics de reciente creación

La estrategia de Valentia Biopharma

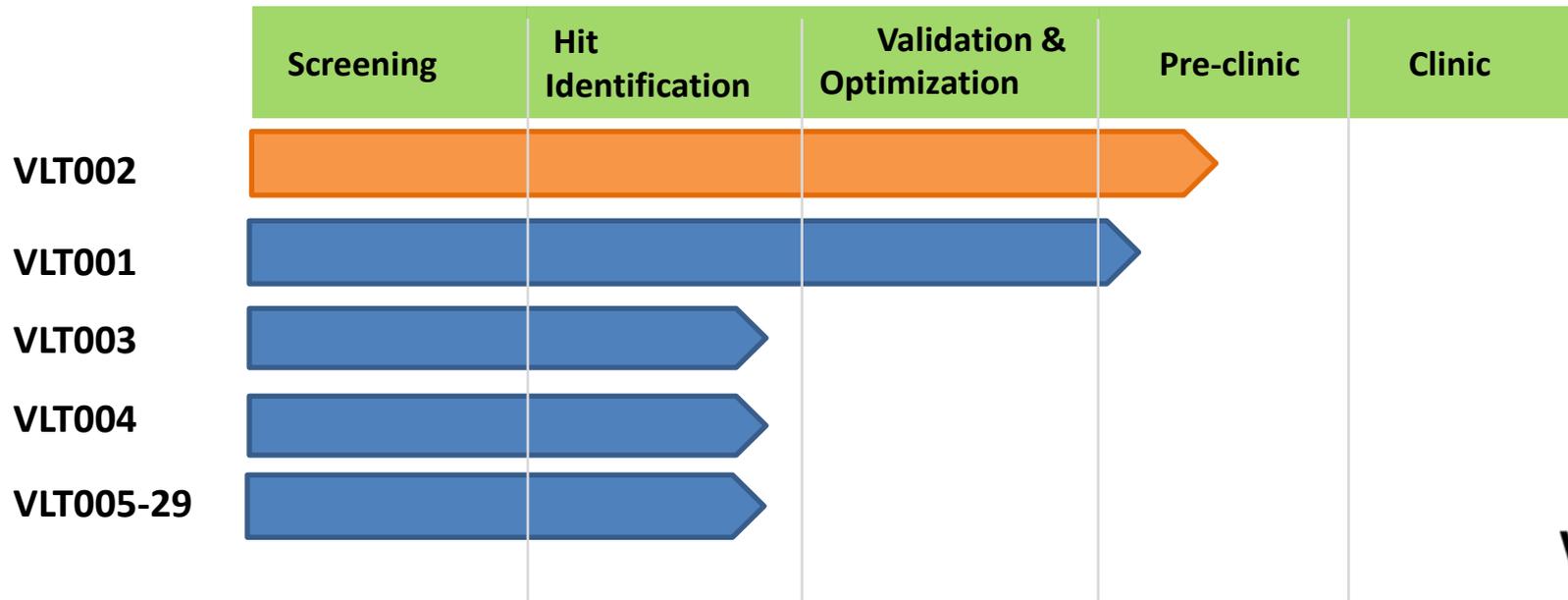


Modelo DM1



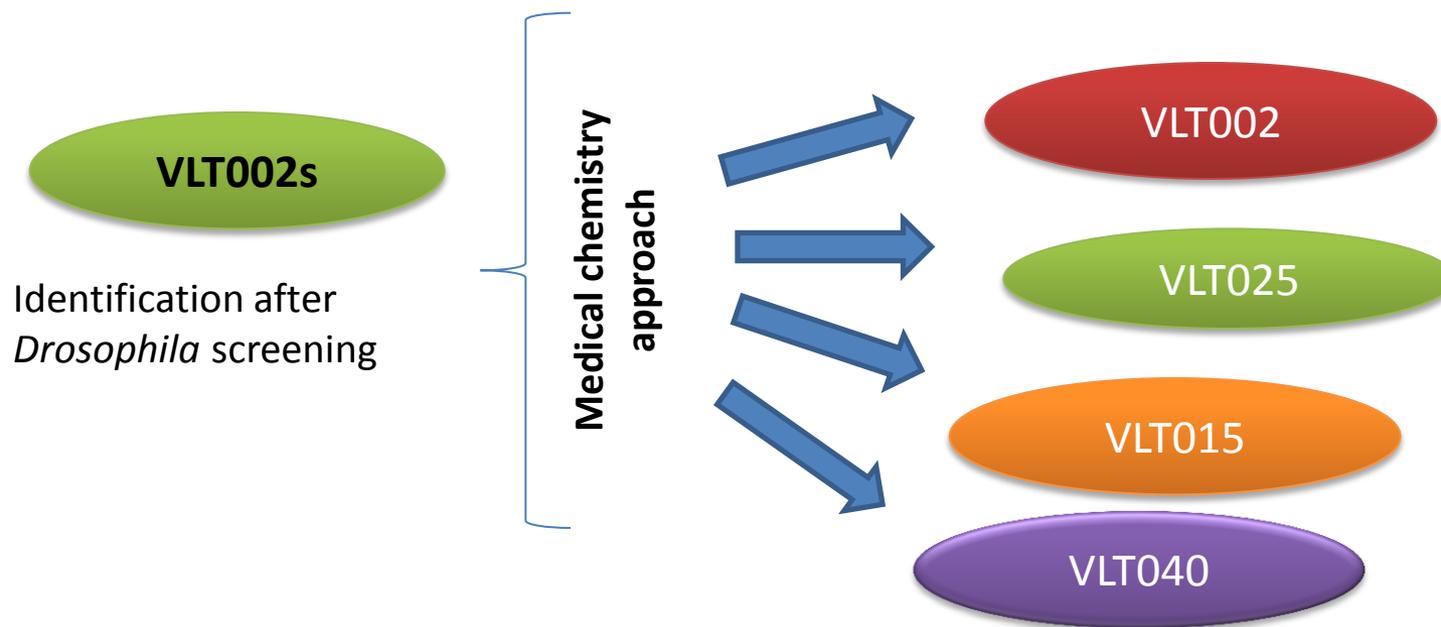
Research

Development



Compuestos VLT002

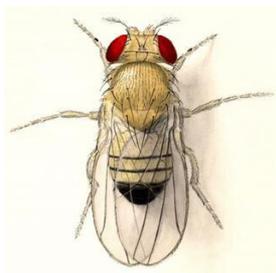
- Familia de 13 compuestos
- Presentación de patente Europea (Diciembre 2013)



Resumen de la actividad de los compuestos de VLT

Altamente eficaces

Mejoran diferentes fenotipos de DM1



Drosophila

- Aumento de área muscular
- Supresión de fenotipos moleculares



Ratón

- Supresión de defectos histológicos musculares
- Supresión de fenotipos moleculares
- Supresión de fenotipos funcionares (reducción de miotonía)



Cultivos celulares humanos

- Supresión de fenotipos moleculares

Resumen de la actividad de los compuestos de VLT

Baja toxicidad

- Por el momento, todos los estudios de toxicidad realizados muestran la baja toxicidad de los compuestos y su amplia ventana terapéutica.

Excelente perfil farmacocinético

- Efecto terapéutico dependiente de dosis
- Gran ventana terapéutica

El futuro de los compuestos VLT002

- Mejora de la solubilidad e identificación del vehículo adecuado
- Medida de la eficacia por vía de administración intravenosa

Plan de desarrollo

2012

3Q2013

3Q 2014

4Q 2014

Estudios
de eficacia
y ADME-
Tox

Preclínica
regulatoria

Dossier de
preclínica
regulatoria

Inicio fase
clínica

Designación
Huérfano

Venta

Ensayos clínicos

The screenshot shows the ClinicalTrials.gov website interface. The search bar contains the text "myotonic dystrophy" and the results show 16 studies found. The first study is "Clinical Efficacy Trial of Mexiletine for Myotonic Dystrophy Type 1" (Recruiting) and the second is "Postural Spirometry Changes in Ambulatory Myotonic Dystrophy Patients" (Completed). The website header includes the logo "ClinicalTrials.gov" and the text "A service of the U.S. National Institutes of Health". The search bar also includes a search button and a "Search" button. The results table has columns for Rank, Status, and Study. The first study is ranked 1 and is in the "Recruiting" status. The second study is ranked 2 and is in the "Completed" status. The website also features a navigation menu with options like "Find Studies", "About Clinical Studies", "Submit Studies", "Resources", and "About This Site".

www.clinicaltrials.gov

Solo compuestos paliativos: mexiletine, DHEA, IPLEX (rhIGF-I/rhIGFBP-3)

Valentia Biopharma



- 9 profesionales, mayoría con perfil científico
- Instalaciones de mas de 100m²

Noticias sobre investigación en DM1

Foro del paciente

www.valentiabiopharma.com

• Página principal x

www.valentiabiopharma.com/foro/

VLT BIOPHARMA

FAQ BUSCAR REGISTRARSE IDENTIFICARSE IR A VALENTIA BIOPHARMA

Índice general

Buscar temas sin respuesta • Ver temas activos

FORO	TEMAS	MENSAJES	ÚLTIMO MENSAJE
Información General Información general sobre enfermedades raras.	0	0	No hay mensajes
Distrofia Miotónica Preguntas y respuestas sobre la Distrofia Miotónica	5	17	por MCarmenVLT Lun, 25 Feb 2013, 10:01
Drosophila como modelo in vivo La mosca <i>Drosophila melanogaster</i> como modelo para el descubrimiento de nuevos fármacos. Moderador: valentiaweb	1	3	por valentiaweb Mar, 30 Oct 2012, 15:41
Pregunta a nuestros expertos Consulta todas las dudas específicas de tu caso con nuestro equipo de investigadores. Moderador: valentiaweb	5	12	por MCarmenVLT Mar, 26 Feb 2013, 12:57

IDENTIFICARSE • REGISTRARSE

Nombre de Usuario: Contraseña: | Identificarse automáticamente en cada visita

¿QUIÉN ESTÁ CONECTADO?

Hay 3 Usuarios identificados :: 0 registrado, 0 ocultos y 3 invitados (basados en usuarios activos en los últimos 5 minutos)

ES 13:54 11/04/2013

Noticias sobre investigación en DM1

Facebook

www.facebook.com/distrofiamiotonica

The screenshot shows the Facebook page for 'Distrofia Miotónica'. The page header includes the name 'Distrofia Miotónica' and the user 'MCarmen Alvarez'. The main content area features a large green banner with the text 'Investigación en Distrofia Miotónica' and the website 'www.valentiabiopharma.com'. Below the banner, there is a section titled 'Distrofia Miotónica' with a 'Te gusta' button and a notification that 461 people like the page. The right sidebar shows a list of users who have interacted with the page, including Jose Sánchez Pascual and Alex Vaquero. The bottom of the page shows a Windows taskbar with various application icons and a system clock indicating 13:59 on 11/04/2013.



Gracias por vuestra atención!

www.valentiabiopharma.com