

Problemas de interpretación de los resultados genéticos en la práctica clínica

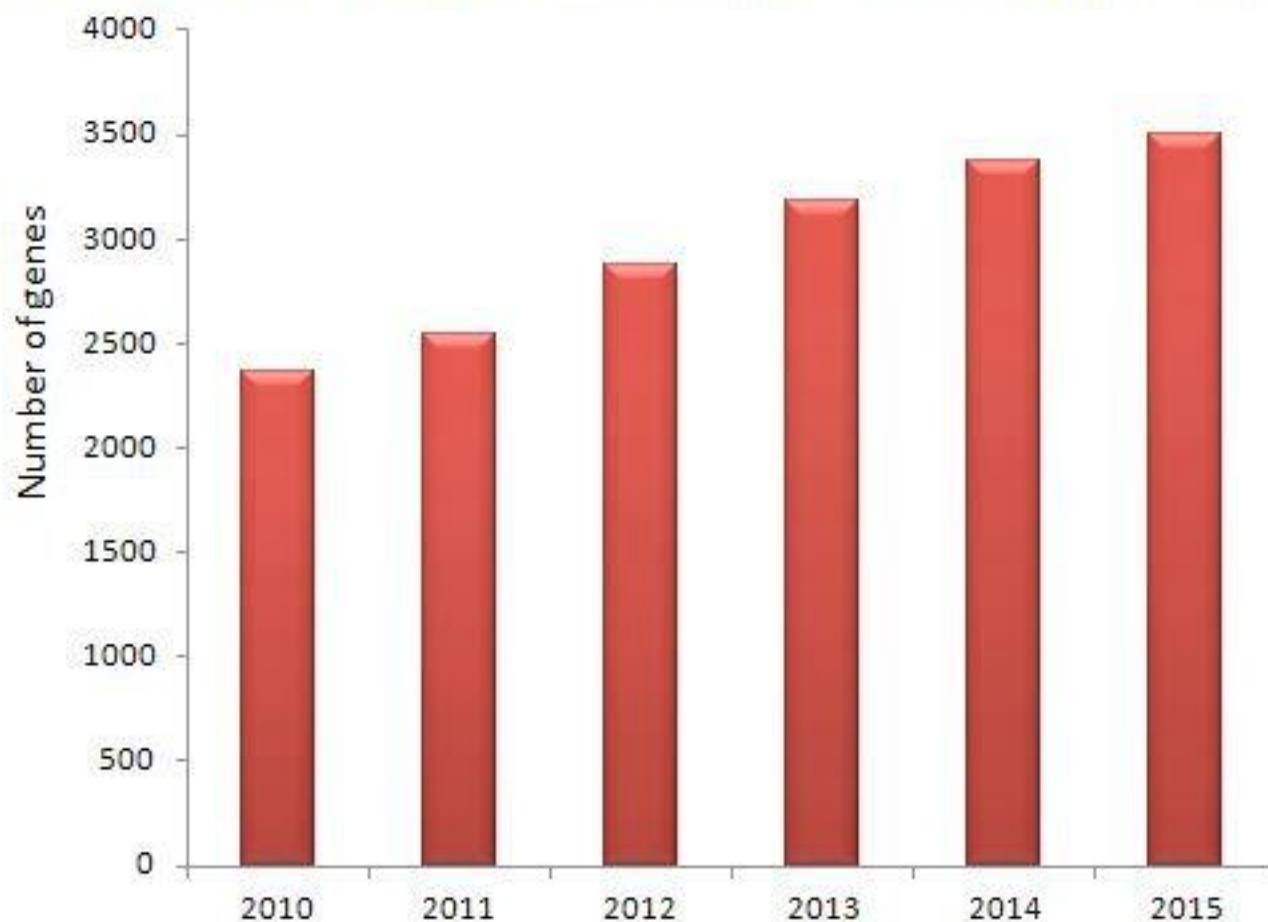
SUMARIO

- ***Consideraciones generales***
 - *El neurologo frente a la interpretación de los resultados moleculares*
 - *Rotura de paradigmas clásicos de la biología molecular*
 - *Técnicas: Sanger, NGS (Paneles, Targeted sequencing)*
 - *El problema de las VUS*
 - *Errores técnicos (falsos negativos y positivos)*
 - *Necesidad de estudios de expresión (transcriptómica y proteómica)*
- ***Situación actual de las principales miopatías***
- ***El problema de las dobles/triples... mutaciones: el caso de la TTN***
- ***Algunas consideraciones finales***

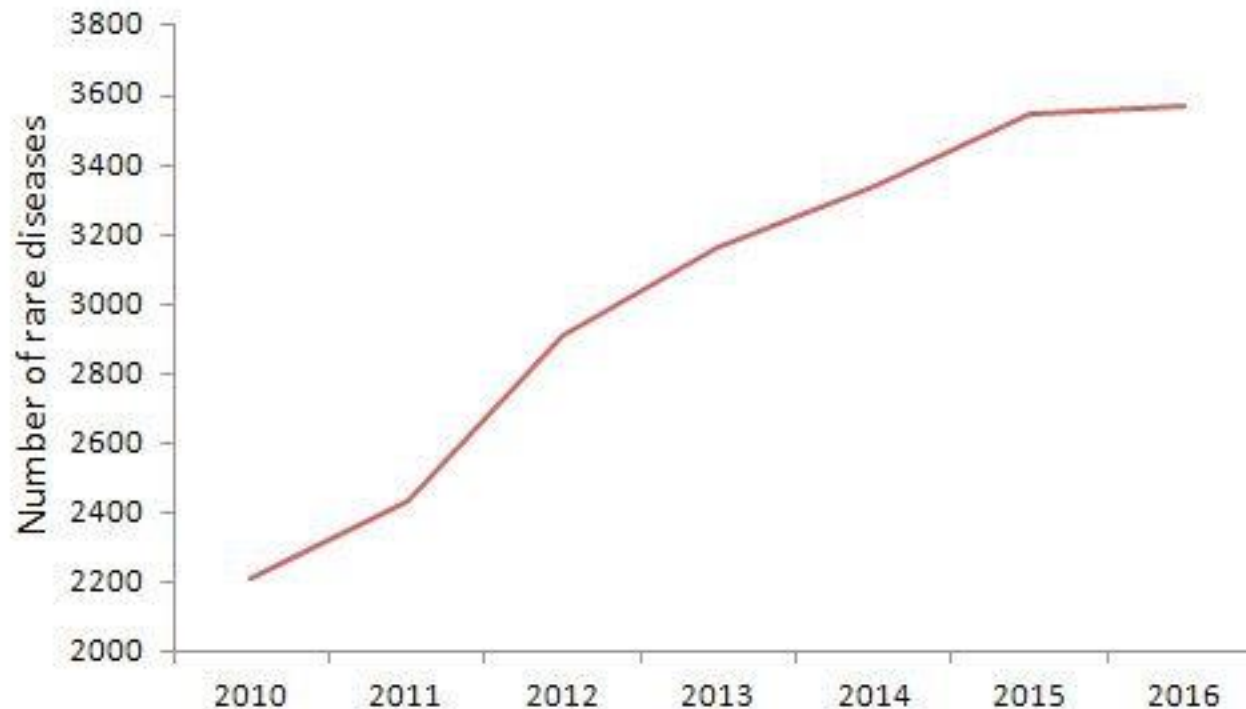
POR QUÉ LOS CLINICOS DEBEN CONOCER LOS ENTRESIJOS DEL DIAGNOSTICO MOLECULAR

- Para comprender mejor la fisiopatología de las enfermedades de base genética
- Para entender las bases de las variaciones fenotípicas y poder explicar mejor lo que ocurre a nuestros pacientes
- Para poder seleccionar grupos homogéneos de cara a los ensayos clínicos
- Para poner en valor los resultados de los estudios moleculares complejos y/o adoptar estrategias de diagnóstico alternativo
- Porque la competencia global sobre el diagnóstico (clínico), consejo genético y el tratamiento de estas enfermedades es una **competencia clínica de cada especialidad** (perfiles) frente a otras opciones que están en la agenda

Cumulative number of genes linked to rare diseases by year since 2010

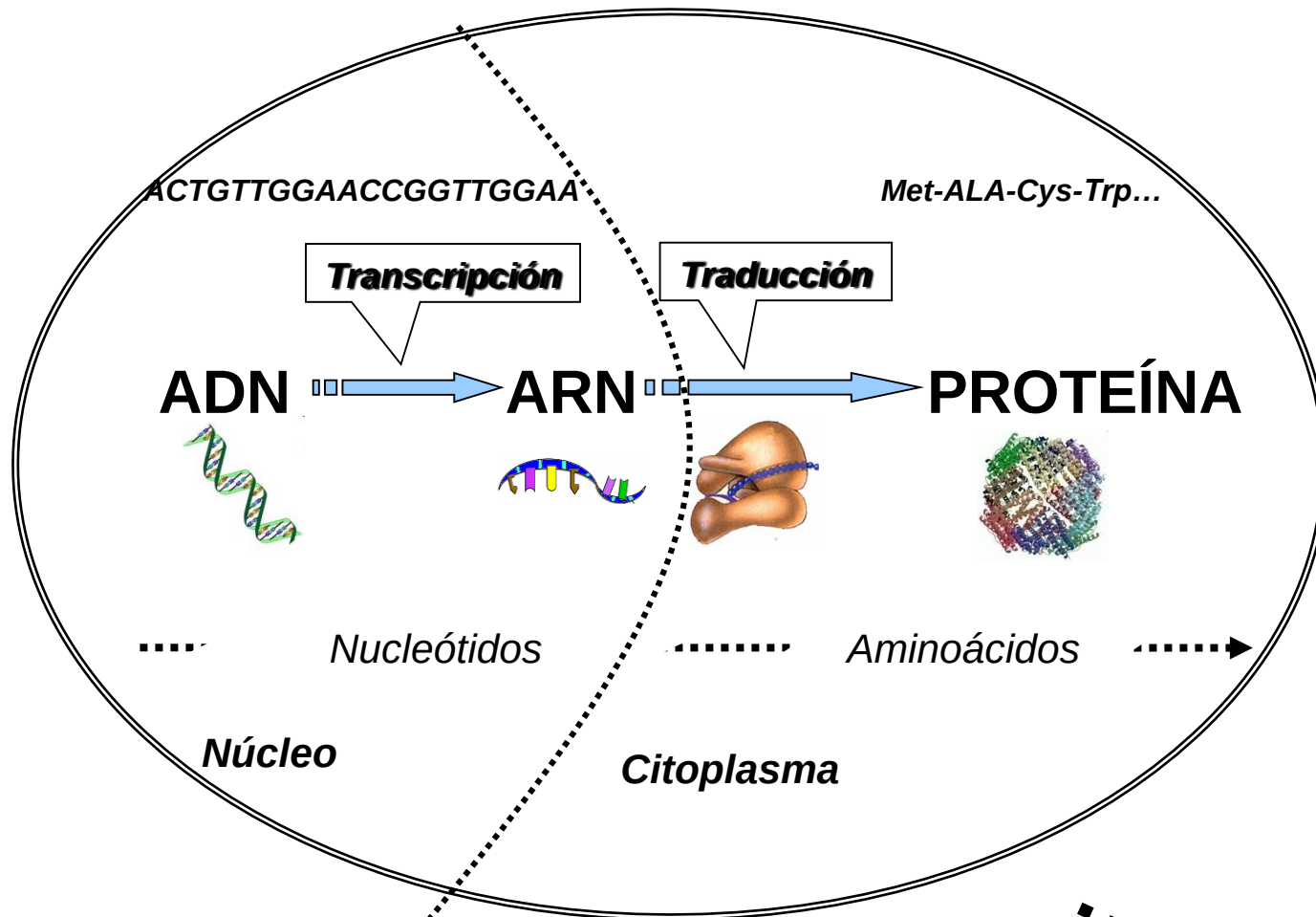


N° of RD for which there is a genetic test available according to Orphanet data*



*Australia, Austria, Belgium, Bulgaria, Canada, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Israel, Italy, Latvia, Lebanon, Lithuania, Macedonia, Morocco, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Switzerland, Sweden, Tunisia, Turkey, Ukraine, United Kingdom

EL ESCENARIO DEL DIAGNOSTICO MOLECULAR



PARADIGMAS TRADICIONALES

UN GEN → UN ARN → UNA PROTEÍNA

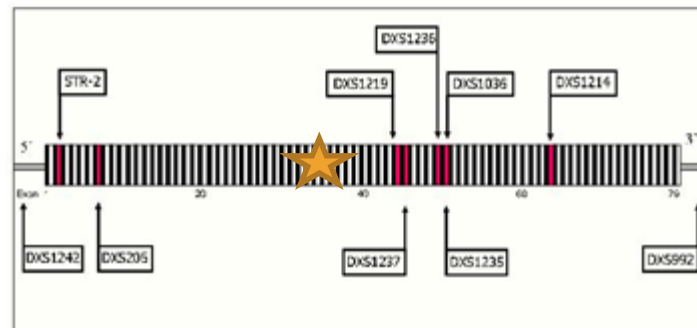
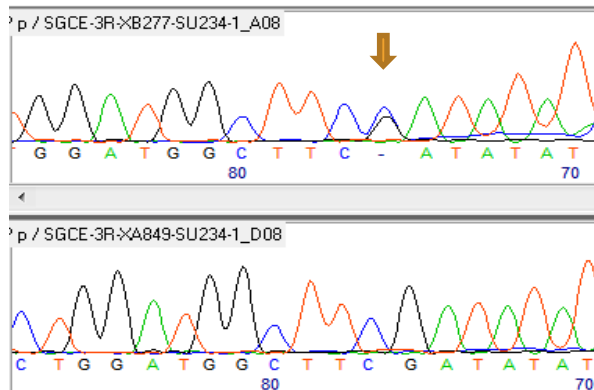
UNA PROTEÍNA → UNA FUNCIÓN

UNA FUNCIÓN → UN FENOTIPO

¡¡¡FALSO!!!

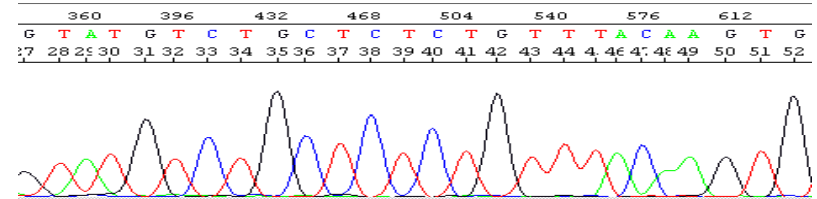
Tipos de pruebas genéticas

- a. **Alteraciones cromosómicas: Pruebas citogenéticas**
- b. **Alteraciones genéticas: Pruebas moleculares**
 - i. **directas (mutación causal)**
 - i. **Sanger (cambios nucleotidos, pequeñas deleciones)**
 - ii. **MLPA (grandes deleciones o reordenamientos)**
 - iii. **Mutaciones dinámicas (expansiones)**
 - ii. **indirectas (haplotipo asociado a la enfermedad)**
- c. **Alteraciones RNA/miRNAs/lncRNAs, etc..**
- d. **Alteraciones proteína (Western Blot y fenomenos postraduccionales)**
- e. **Alteraciones epigenéticas (metilación, acetilación, sumoylación,...)**



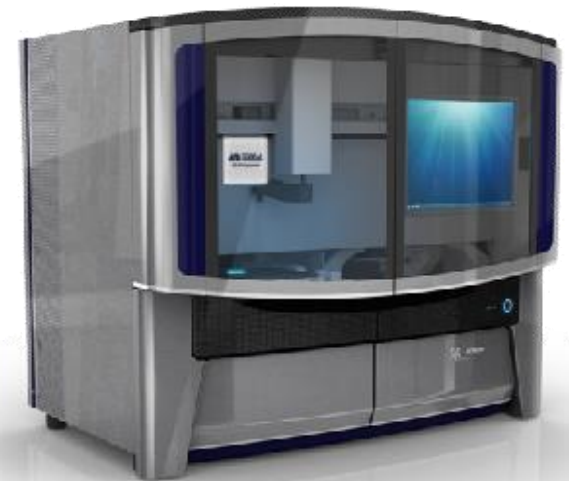
ULTRASECUENCIACIÓN (NEXT GENERATION SEQUENCING)

Secuenciación estándar:
gen a gen (selección en base a la sospecha clínica)



Ultrasecuenciación:

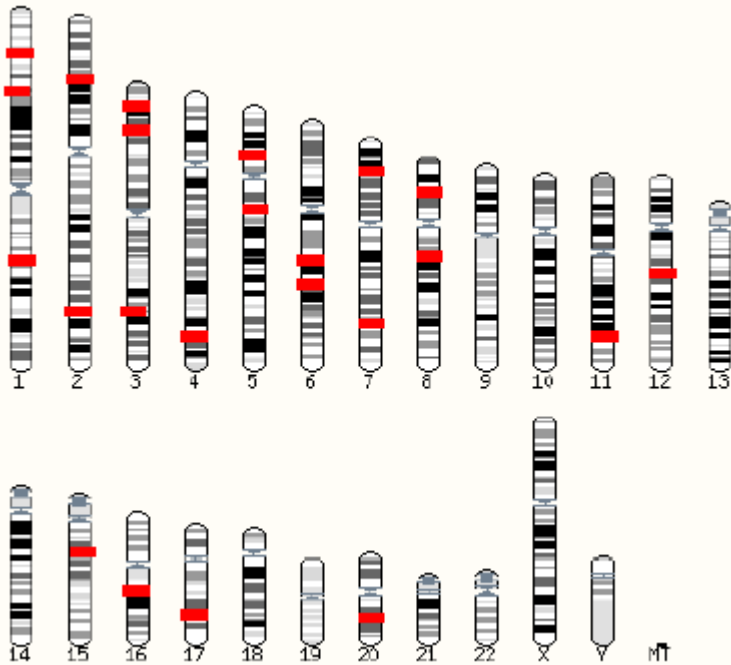
- genes relacionados con una patología determinada: **“Targeted resequencing”**.
- todos los genes simultáneamente: **“Exome Sequencing”**
- Todo el genoma: **“Whole Genome Sequencing”**
- Precio 2018 Exoma 400-500 Euros y **bajando...**



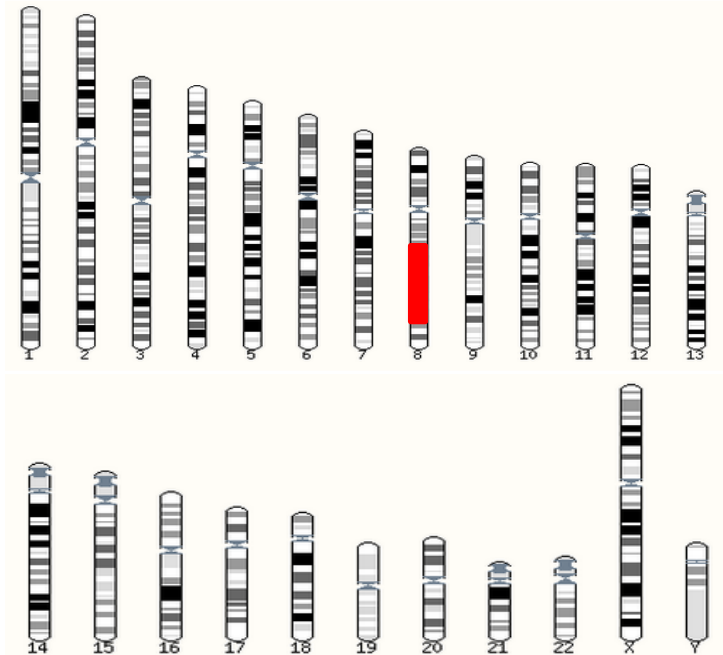
Distintos tipos de aproximaciones

Targeted Resequencing

Zonas o genes seleccionados



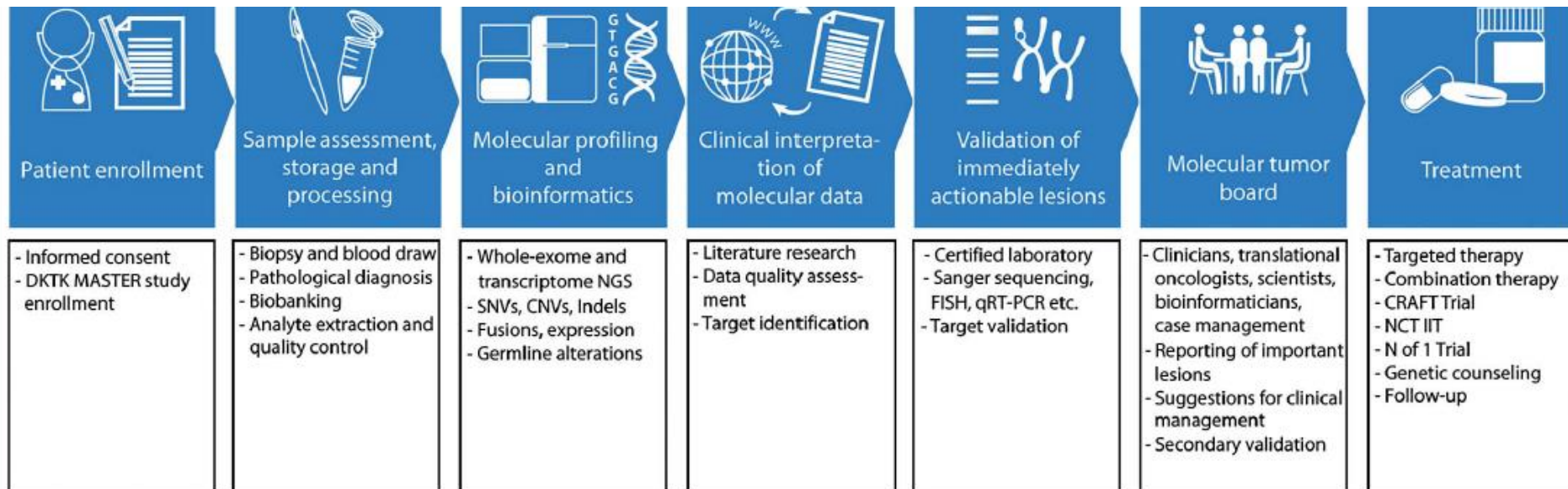
Paneles de genes específicos de una patología o grupo de patologías



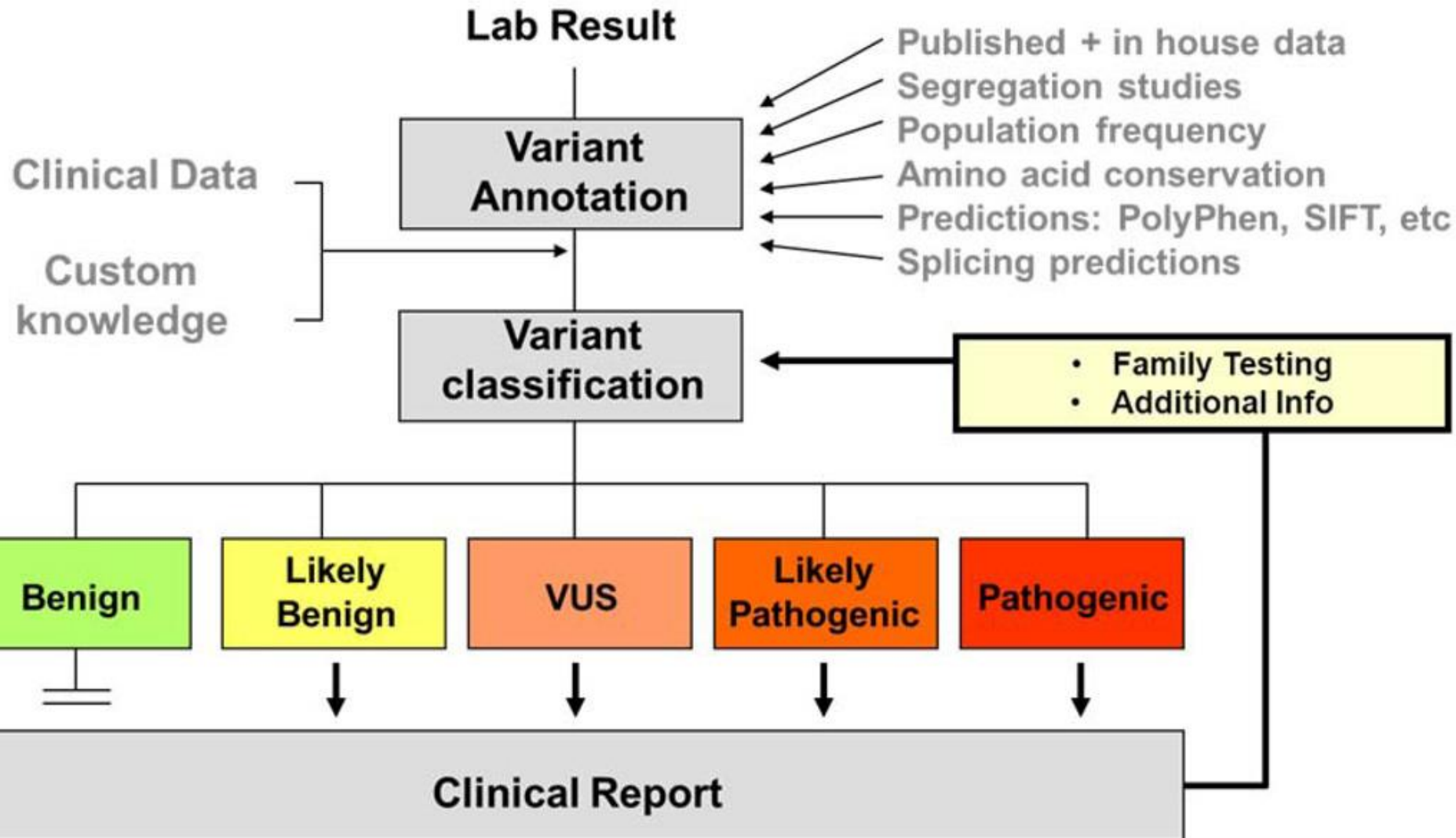
Secuenciación de una región de interés

	Targeted panels	Whole exome	Whole genome
Pro	▶ High depth of coverage ▶ Readily standardisable ▶ Rapid interpretation for clinical use ▶ Low costs ▶ Easy clinical implementation	▶ Detection of unknown variants ▶ Detection of CNVs ▶ Research applications ▶ Feasible in clinical routine ▶ Low price/performance ratio	▶ Comprehensive assessment of cancer genomes ▶ Highest resolution of genomic alterations ▶ SNVs in enhancer/promoter and ncRNA regions ▶ Decreasing costs ▶ Subject to future studies
Contra	▶ Limited, 'peephole' observations ▶ Limited value for research ▶ Limited assessment of complex aberrations	▶ Not fully comprehensive ▶ Lower CNV resolution ▶ Amplification or exon capture necessary ▶ High bioinformatic effort ▶ Demanding clinical interpretation ▶ Time-consuming workflow	▶ Uncertain value for clinical interpretation ▶ Most expensive

Integrating next-generation sequencing



FLUJO DE TRABAJO



Parlez-vous VUS?



The banner features a light gray background with a faint image of a family. On the left, the 'FindMyVariant' logo is displayed, consisting of a stylized tree with colorful leaves and a heart shape. To the right of the logo are the words 'FindMy' in a small font and 'VARIANT' in a large, bold font. Further right are three links: 'WHAT IS A VUS', 'OUR GOALS', and 'EXAMPLES'. Below the logo, three lines of text are presented: 'Did your genetic testing identify a Variant of Uncertain Significance (VUS)?', 'Do you want more information on what this variant means for you and your family?', and 'With your family's help you can discover more about your genetic variant.' To the right of the text is a family tree diagram made of orange lines and circles, with several circular photos of people from different generations. At the bottom, a red banner contains a location pin icon with a DNA helix inside, followed by the text 'Find your variant in your family.' and a yellow button with the text 'Get Started' and a right-pointing arrow.

**FindMy
VARIANT**

WHAT IS A VUS OUR GOALS EXAMPLES

Did your genetic testing identify a Variant of Uncertain Significance (VUS)?

Do you want more information on what this variant means for you and your family?

With your family's help you can discover more about your genetic variant.

 Find your variant in your family.

Get Started >

While family studies have the potential to yield powerful data to reclassify variants, the time and effort required to build pedigrees adequately for variant classification limit this potential. We developed an educational website containing a patient-friendly toolkit to help aid patients in working with research and clinical laboratories to reclassify their own VUS.

Table 2. Overview of the different reasons that *SCN1A* mutations were missed in a genetic screening.

Patient	Negative screening	Positive screening	Reason that the mutation was missed
Human error			
1	Sanger	NGS: WES	Missed by the person performing the mutation analysis (visual inspection of Sanger traces)
2 ¹	Sanger	NGS: Gene panel	Missed by the person performing the mutation analysis (visual inspection of Sanger traces)
3	Sanger	NGS: Gene panel	Missed by the person performing the mutation analysis (visual inspection of Sanger traces)
4	Sanger	NGS: WES	Missed by the person performing the mutation analysis (visual inspection of Sanger traces)
5	Sanger	NGS: WES	Missed by the person performing the mutation analysis (visual inspection of Sanger traces)
6	Sanger	NGS: Gene panel	Missed by the person performing the mutation analysis (visual inspection of Sanger traces)
7	Sanger	NGS: Gene panel	Missed by the person performing the mutation analysis (visual inspection of Sanger traces)
8	Sanger	NGS: Gene panel	Missed by the person performing the mutation analysis (visual inspection of Sanger traces)
9	Sanger	NGS: Gene panel	Missed by the person performing the mutation analysis (visual inspection of Sanger traces)
10	Sanger	NGS: WES	Error in the primer design: The mutation was located in the primer binding site
11	Sanger	NGS: WES	Error in the primer design: A polymorphism in the primer led to mono-allelic amplification
12	Sanger	NGS: Gene panel	Error in the primer design: A polymorphism in the primer led to mono-allelic amplification
13	Sanger	NGS: WES	Error in the primer design: The primer did not cover the whole amplicon (only one direction was sequenced)
14	Sanger	NGS: WES	The patient turned out not to be sequenced
15	Sanger	NGS: WES	Possible sample swap outside the lab
16 ²	Sanger	NGS: Gene panel	Wrong sequencing data assigned to the patient
17 ³	Sanger	NGS: WES	The traces were of bad quality so the Sanger should have been redone
18 ²	Sanger	NGS: Gene panel	Not reported in the diagnostic report as the mutation is located eight base pairs in the intron
19 ¹	Sanger	NGS: Gene panel	An adjacent intronic polymorphic deletion led to misalignment of the alleles and uninterpretable data
Technical error			
20 ⁴	Sanger	NGS: WES	The annealing temperature of the primers was too high for the polymerase
21	Sanger	NGS: WES	The mutated peak was too low
22	NGS: WES	Sanger	The mutation is located in a homopolymer stretch
Unknown reason			
23	Sanger	NGS: WES	Unable to retrieve the Sanger traces
24	Sanger	NGS: WES	Unable to retrieve the Sanger traces
25	Sanger	NGS: Gene panel	Unable to retrieve the Sanger traces
26	Sanger	NGS: Gene panel	Unable to retrieve the Sanger traces
27	Sanger	NGS: WES	Unable to retrieve the Sanger traces
28	Sanger	NGS: Gene panel	Unable to retrieve the Sanger traces

Pitfalls in genetic testing

Molecular Genetics & Genomic Medicine

Table 1. Genetic and clinical information of the *SCN1A* mutations reported in this study as well as the setting and the date the samples were screened.

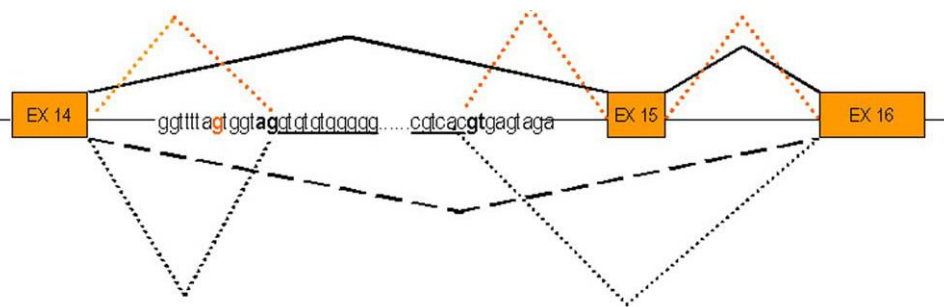
Patient	Mutation		Inheritance	Novel ⁶	Phenotype	Setting	Setting	Date	Date
	cDNA	Protein				negative screening	positive screening	negative screening	positive screening
1 ¹	c.1121C>A	p.Ser374Tyr	<i>De novo</i>	Yes	DS	Research	Research	2012	2012
2 ²	c.664C>T	p.Arg222*	<i>De novo</i>	No	DS	Research	Research	2006	2012
3	c.4002+1G>A		<i>De novo</i>	No	DS	Diagnostic	Diagnostic	2013	2014
4	c.4284+1G>A		<i>De novo</i>	No	DS	Diagnostic	Research	2007	2013
5	c.1178G>A	p.Arg393His	<i>De novo</i>	No	DS	Diagnostic	Research	2009	2011
6	c.5269G>A	p.Gly1757Arg	<i>De novo</i>	Yes	DS	Diagnostic	Research	2010	2013
7	c.5656C>T	p.Arg1886*	<i>De novo</i>	No	DS	Diagnostic	Research	2010	2013
8	c.53_55delCCA	p.Thr18del	Unknown	Yes	DS	Diagnostic	Diagnostic	2010	2015
9	c.602+1G>C		Unknown	Yes	DS	Diagnostic	Diagnostic	2010	2015
10 ¹	c.5461C>T	p.Gln1821*	<i>De novo</i>	No	DS	Research	Research	2011	2013
11	c.379C>T	p.His127Tyr	<i>De novo</i>	Yes	GEFS+	Diagnostic	Research	2007	2013
12	c.302G>A	p.Arg101Gln	<i>De novo</i>	No	DS	Diagnostic	Diagnostic	2013	2015
13 ¹	c.4853-1G>A		<i>De novo</i>	Yes	DS	Research	Research	2011	2013
14 ¹	c.3439G>T	p.Glu1147*	<i>De novo</i>	No	DS	NA	Research	NA	2012
15	c.302G>A	p.Arg101Gln	<i>De novo</i>	No	DS	Diagnostic	Research	2007	2013
16 ³	c.5195C>T	p.Pro1732Leu	<i>De novo</i>	No	DS	Diagnostic	Research	2010	2013
17 ⁴	c.2044-1G>A		<i>De novo</i>	No	DS	Research	Research	2002	2011
18 ^{3,1}	c.2590-8T>G		<i>De novo</i>	No	DS	Diagnostic	Research	2010	2013
19 ²	c.1178G>A	p.Arg393His	<i>De novo</i>	No	DS	Diagnostic	Research	2009	2011
20 ^{5,1}	c.3452C>G	p.Ser1151*	<i>De novo</i>	No	DS	Research	Research	2010	2012
21	c.4889T>G	p.Val1630Gly	<i>De novo</i>	Yes	DS	Diagnostic	Research	2011	2013
22	c.2589+3A>T		<i>De novo</i>	No	DS	Research	Research	2013	2005
23 ¹	c.4786C>T	p.Arg1596Cys	<i>De novo</i>	No	DS	Diagnostic	Research	2008	2013
24 ¹	c.5347G>A	p.Ala1783Thr	<i>De novo</i>	No	DS	Research	Research	2010	2012
25	c.5536_5539delAAAC	p.Lys1846Serfs*11	Unknown	No	Unspecified EE	Diagnostic	Research	2008	2012
26	c.5771delG	p.Arg1924Leufs*8	<i>De novo</i>	Yes	DS	Diagnostic	Diagnostic	2014	2014
27	c.4573C>T	p.Arg1525*	<i>De novo</i>	No	MAE	Diagnostic	Diagnostic	2006	2013
28	c.1129C>T	p.Arg377*	Absent in mother, father not tested	No	DS	Research	Research	2004	2013
29 ⁴	c.383C>A	p.Ser128*	<i>De novo</i>	No	DS	Diagnostic	Diagnostic	2011	2011

LIMITACIONES DE LOS ESTUDIOS DE SECUENCIACIÓN (SANGER O NGS)

- No encontrar hallazgos significativos tras la secuenciación de un gen determinado, **no excluye casi nunca al 100% que no existan mutaciones en ese gen** (depende de la cobertura del NGS)
- Además, Podría haber una mutación **fuera de las regiones estudiadas como las zonas intrónicas o zona promotora**
- O tratarse de un tipo de mutación que no puede ser identificado por secuenciación (gen a gen/NGS), **como grandes deleciones o inserciones que pueden afectar parcial o totalmente al gen.**

Characterization of novel *CAPN3* isoforms in white blood cells: an alternative approach for limb-girdle muscular dystrophy 2A diagnosis

A)



(A)

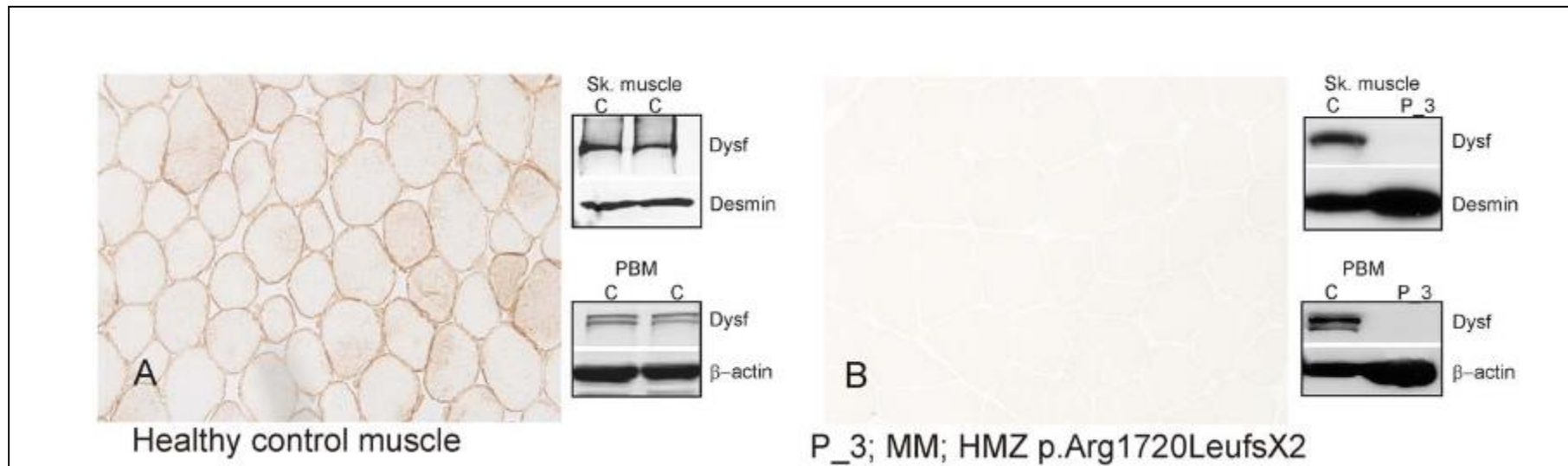
Group	Mean Value (± SD)
Controls (n=2)	1,00 ± 0,12
Patient 19	0,90 ± 0,07
Patient 20	1,08 ± 0,08
Patient 22	1,02 ± 0,11

(B)

Group	Relative Expression (Mean ± SD)
Controls (n=2)	1.00 ± 0.02
Patient 19	0.18 ± 0.01
Patient 20	0.62 ± 0.03
Patient 22	0.11 ± 0.01

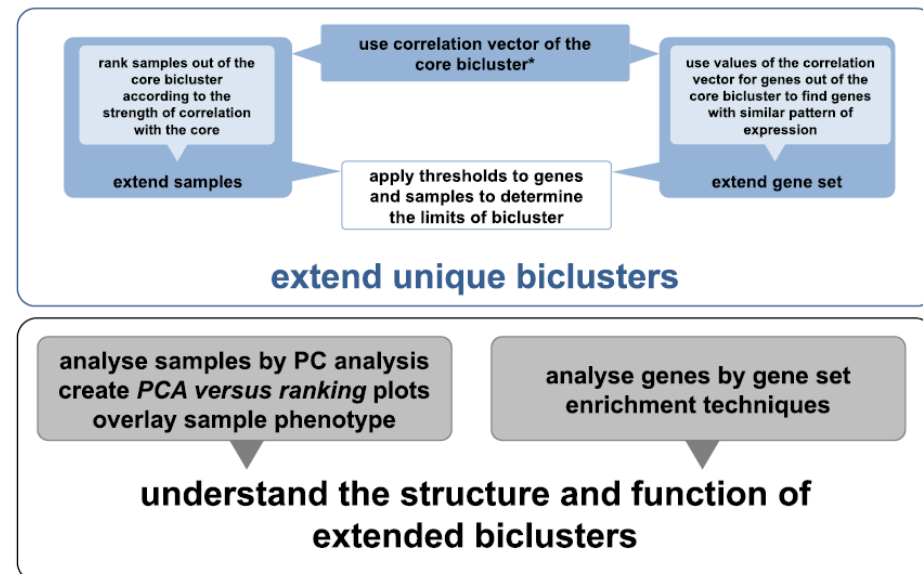
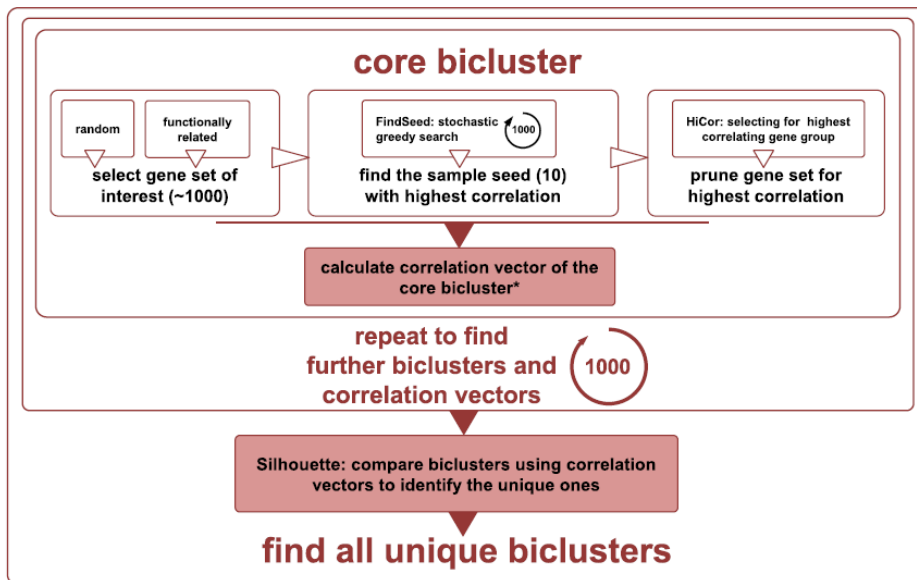
O A NIVEL DE LA PROTEÍNA

- LGMD2B: Enfermedad muscular recesiva causada por mutaciones en el gen que codifica para la proteína **Disferlina (230kda)** (*Liu et al., 1998*).
- Función: Reparación membrana de la fibra muscular (*Bansal et al., 2003*).
- La disferlina se expresa en músculo y monocitos CD14+ (*Gallardo et al., 2011*).
- Existe una correlación de la expresión disferlina en músculo y PBMCs por lo que es útil para orientar el diagnóstico molecular (*Ankala et al., 2014*).
- Sin embargo, existe déficit de disferlina secundarias a otras enfermedades musculares por lo que es necesario confirmar el diagnóstico por secuenciación del del *mRNA DYSF* de los monocitos (*De luna et al., 2001*).



MCbiclust: a novel algorithm to discover large-scale functionally related gene sets from massive transcriptomics data collections

Robert B. Bentham^{1,2,*}, Kevin Bryson^{3,*} and Gyorgy Szabadkai^{1,2,4,*}



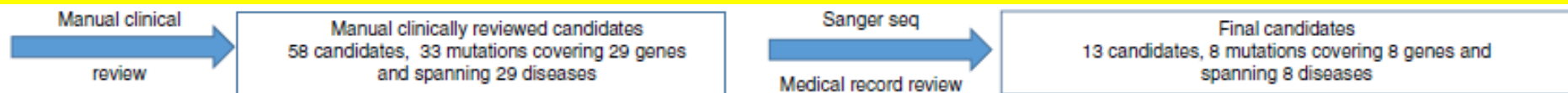
Los 13 humanos que no necesitaban genes esenciales para sobrevivir
El análisis de medio millón de genomas descubre a 13 personas sanas que
deberían estar muertas por falta de algún gen vital; pero la ley impide
identificarlas

MSSM Biobank (N = 11,212)
 CHOP (N = 96,007) Subtotal: 518,721

SWE-SCZ (N = 5,092) TCGA (N = 4,114)
 CHOP-BGI (N = 699) Subtotal: 67,061

1,000 Genomes (N = 1,092)
 UK10K (N = 2,432) Subtotal: 3,524

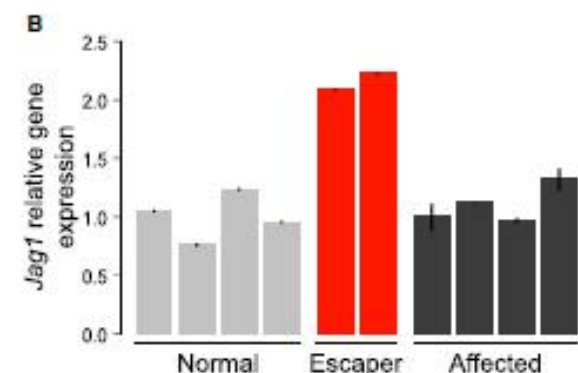
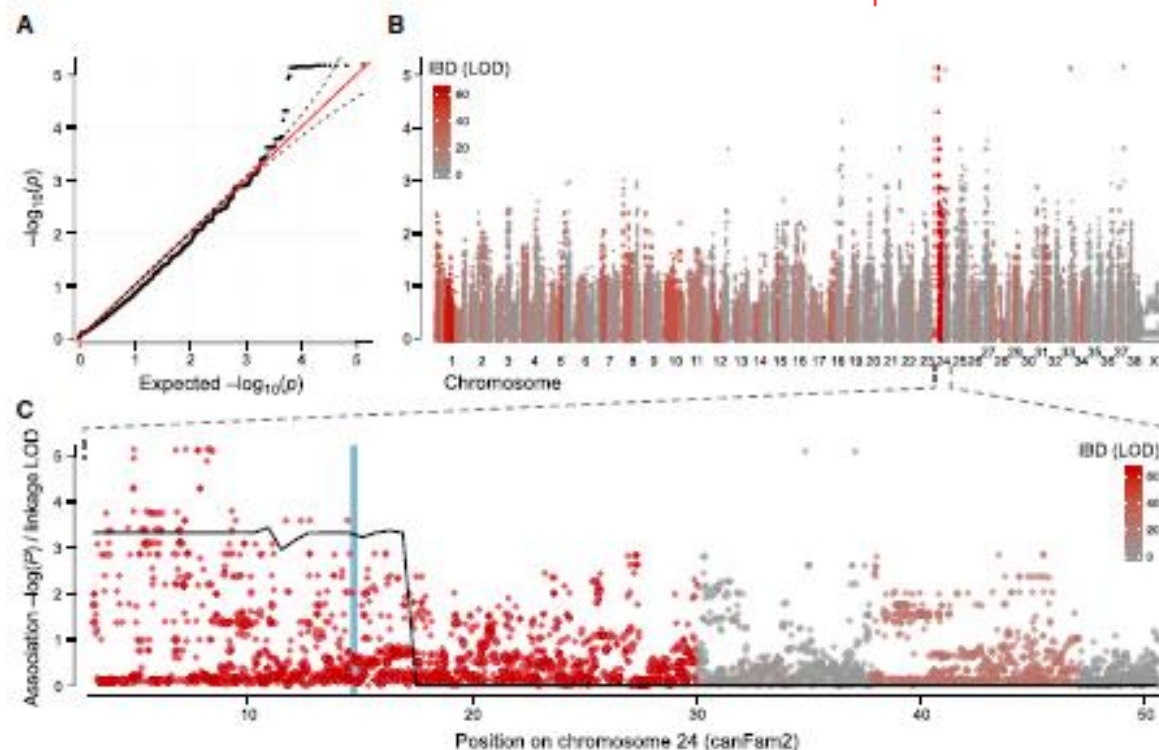
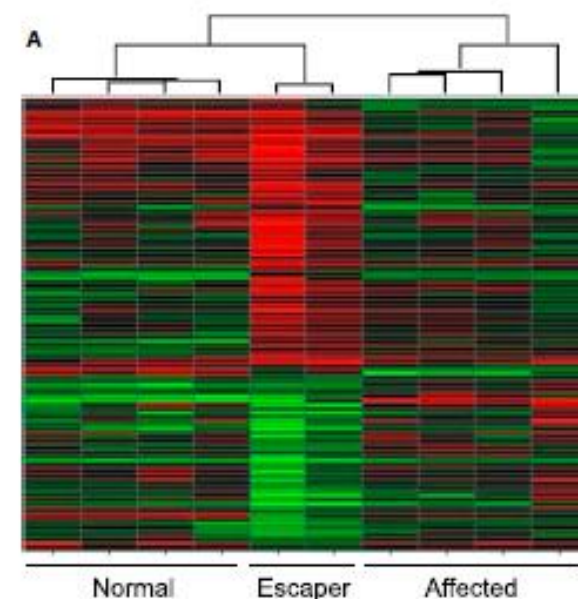
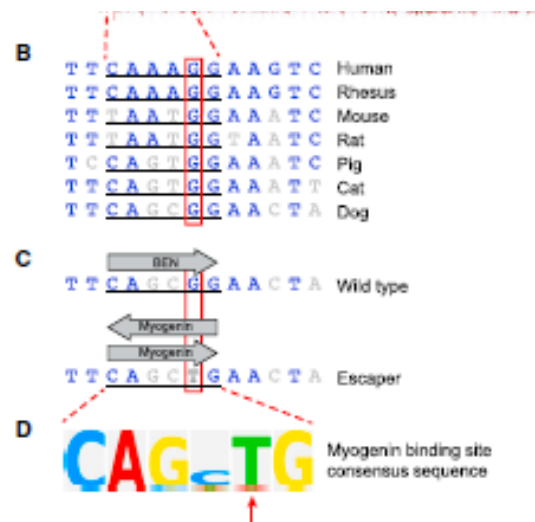
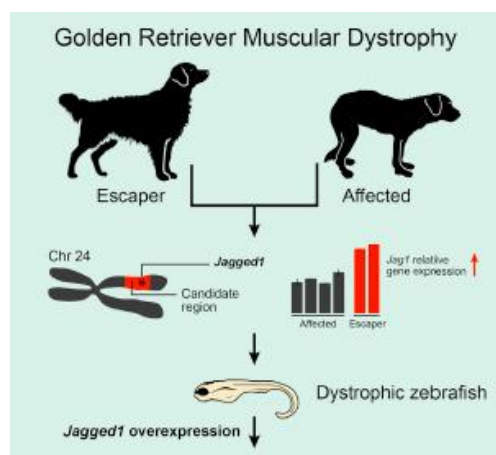
La regulación más específica y detallada se encuentra en la Ley de Investigación Biomédica. ***Existe la obligación de respetar la voluntad del sujeto, al que se le debió consultar antes de que otorgara su consentimiento en relación con la comunicación de hallazgos derivados de un análisis que se vayan a realizar con fines de investigación***, (artículos 59.1 i. de la LIB y 23.2 i. del Real Decreto 1716/2011, de 18 de noviembre, por el que se establecen los requisitos básicos de autorización y funcionamiento de los biobancos con fines de investigación biomédica y del tratamiento de las muestras biológicas de origen humano, y se regula el funcionamiento y organización del Registro Nacional de Biobancos para investigación biomédica). Los resultados relevantes para la salud deben por lo tanto ser comunicados al donante si es que así lo señaló.



Jagged 1 Rescues the Duchenne Muscular Dystrophy Phenotype

Cell 163, 1–10, November 19, 2015 ©2015 Elsevier Inc.

Natassia M. Vieira,^{1,2,3} Ingegerd Elvers,^{4,5} Matthew S. Alexander,^{1,2,6} Yuri B. Moreira,⁷ Alal Eran,² Juliana P. Gomes,³ Jamie L. Marshall,^{1,2} Elinor K. Karlsson,⁴ Sergio Verjovski-Almeida,^{7,8} Kerstin Lindblad-Toh,^{4,5,10} Louis M. Kunkel,^{1,2,9,10,*} and Mayana Zatz^{3,10,*}



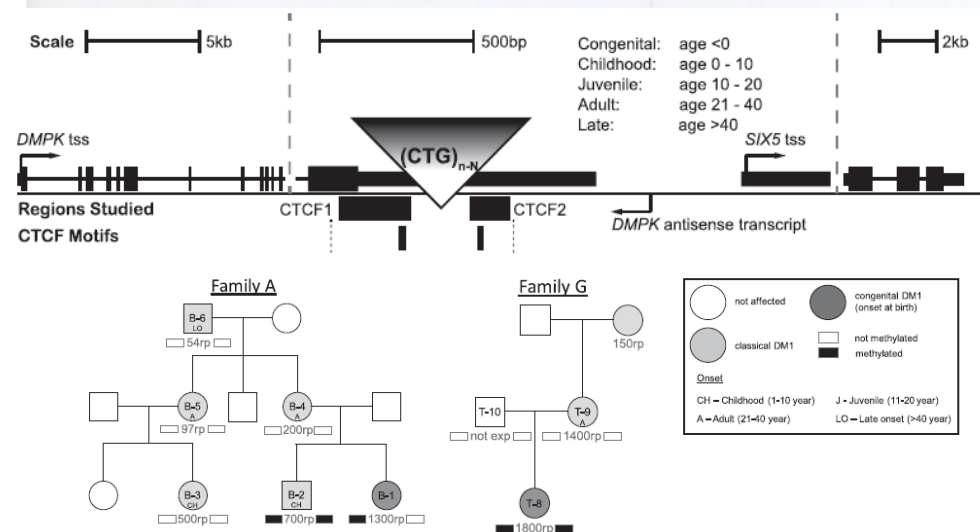
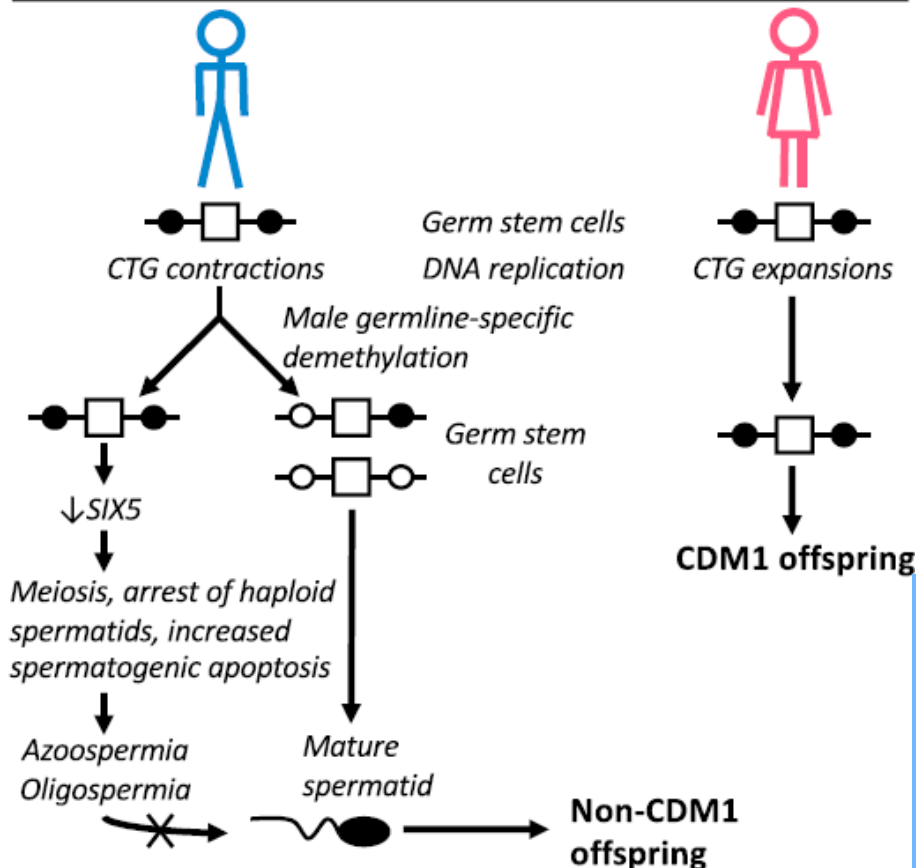
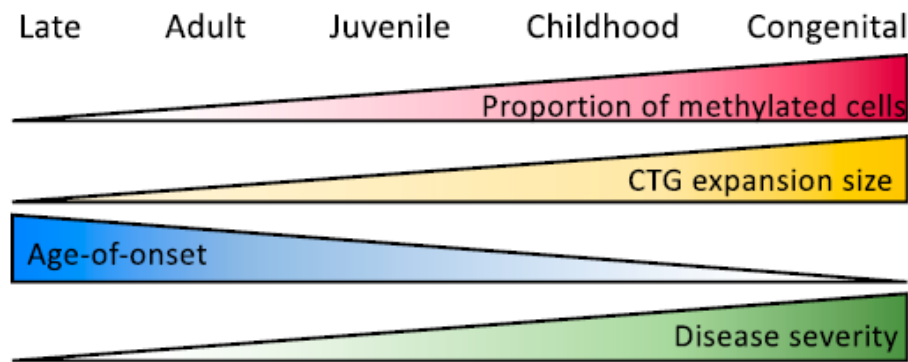
Prevalence of Titin Truncating Variants in General Population

Oyediran Akinrinade¹, Juha W. Koskenvuo^{2,3}, Tero-Pekka Alastalo^{1,2*}

Table 1. Rare variants (MAF <0.002) in the 5 most frequent sarcomere genes, 25 genes associated with or candidate for HCM and 24 genes (same panel excluding *TTN*).

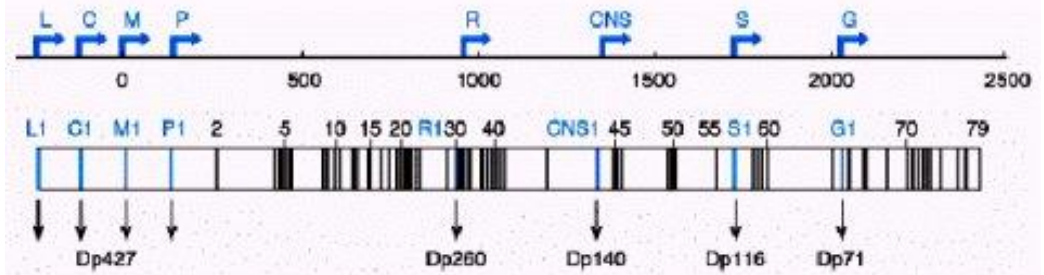
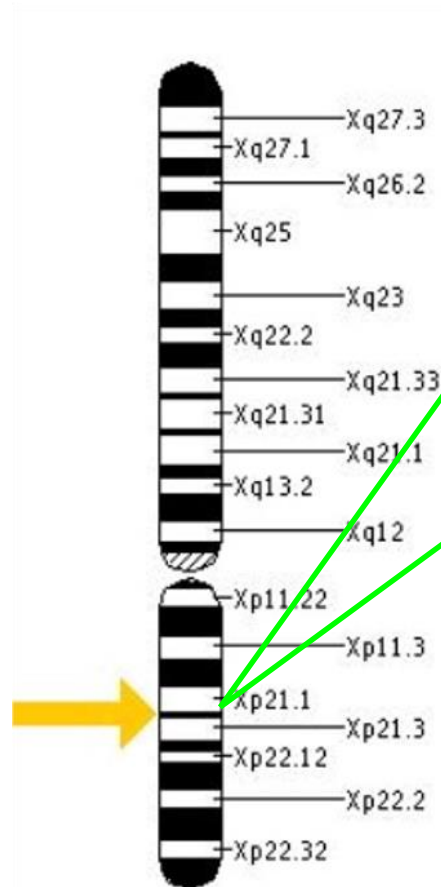
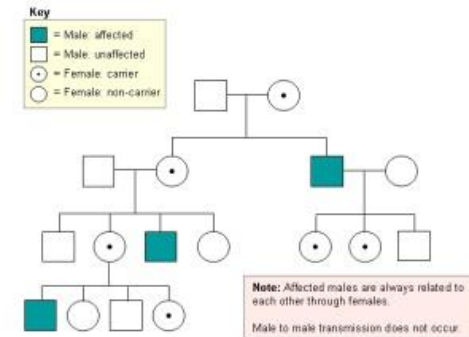
	Main Sarcomere Genes			25 Gene Panel	24 Genes (excluding <i>TTN</i>)
	Pooled Data	Sanger cohort	NGS cohort	NGS cohort	NGS cohort
Patients n	387	84	303	303	303
Positive test n (%)	129 (33.3)	30 (35.7)	99 (32.7)	111 (36.6)	111 (36.6)
Positive test or novel candidates n (%)	130 (33.6)	30 (35.7)	100 (33.0)	121 (39.9)	115 (38.0)
Test with non-benign variants ⁽¹⁾ n (%)	163 (42.1)	35 (41.7)	128 (42.2)	220 (72.6)*	171 (56.4)* [#]
Inconclusive test n (%)	34 (8.8)	5 (6.0)	29 (9.6)	109 (36.0)*	60 (19.8)* [#]
Number of rare variants	187	39	148	398	235*[#]
Pathogenic n (%)	68 (36.4)	14 (35.9)	54 (36.5)	57 (14.3)*	57 (24.3)* [#]
Likely Pathogenic n (%)	67 (35.8)	16 (41.0)	51 (34.5)	60 (15.1)*	60 (25.5) [#]
VUS n (%)	41 (21.9)	6 (15.4)	35 (23.6)	243 (61.1)*	97 (41.3)* [#]
Benign/Likely Benign n (%)	11 (5.9)	3 (7.7)	8 (5.4)	38 (9.5)	21 (8.9)
Novel variants	48	10	38	110*	61[#]
Pathogenic/Likely Pathogenic n (%)	35 (72.9)	9 (90)	26 (68.4)	29 (26.4)*	29 (47.5) [#]
Novel Candidate variants	1 (2.1)	0	1 (2.6)	27 (24.5)*	7 (11.5) [#]
VUS (excluding candidate variants) n (%)	11 (22.9)	1 (10)	10 (26.3)	51 (46.4)*	24 (39.3)
Benign/Likely Benign n (%)	1 (2.1)	0	1 (2.6)	3 (2.7)	1 (1.6)

CpG Methylation, a Parent-of-Origin Effect for Maternal-Biased Transmission of Congenital Myotonic Dystrophy



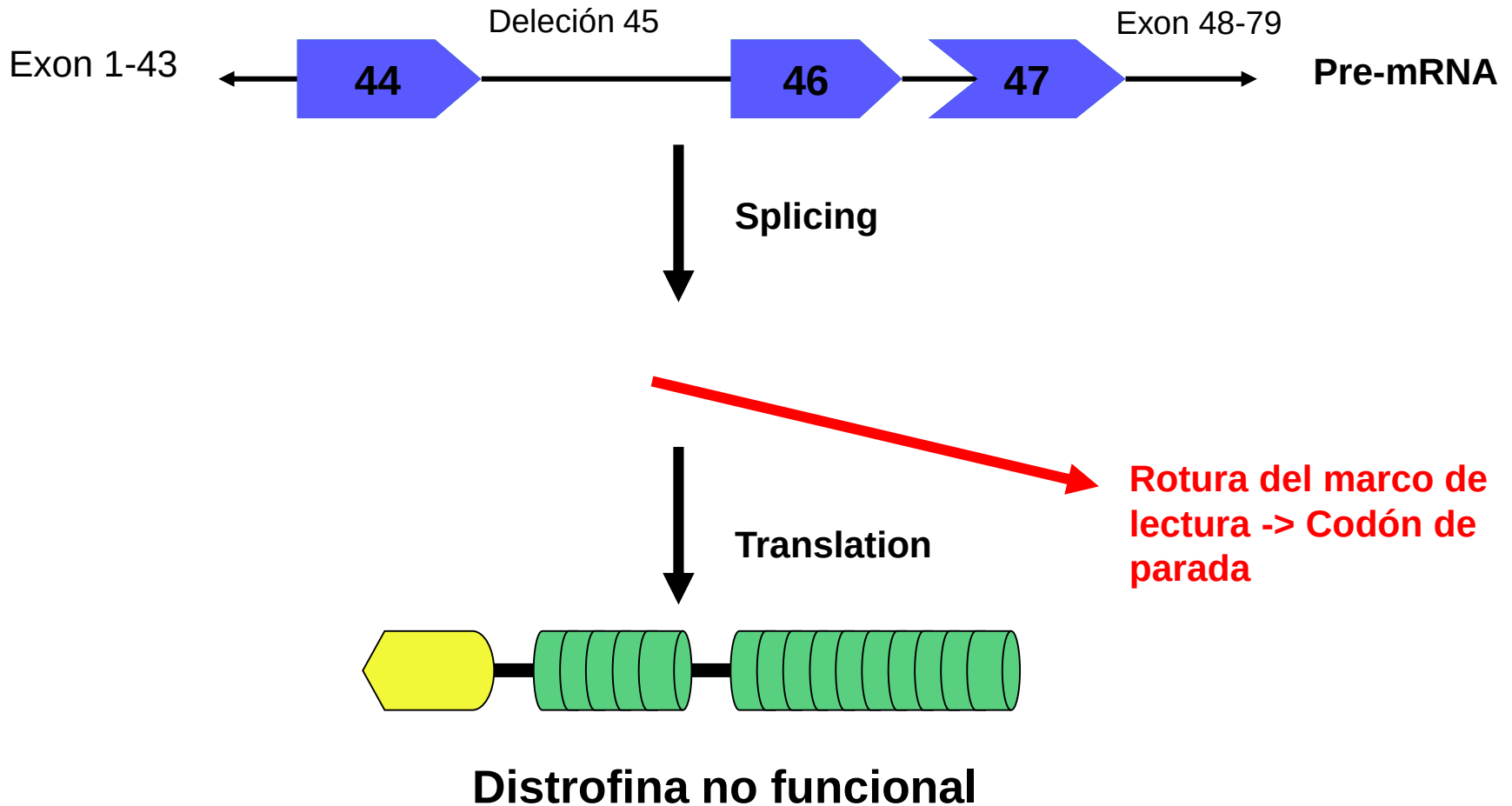
La azoospermia derivada de la metilación intensa de algunas células germinales masculinas las llevaría a su desaparición, eliminando la posibilidad de zigotos altamente metilados por vía paterna

DISTROFINOPATIAS

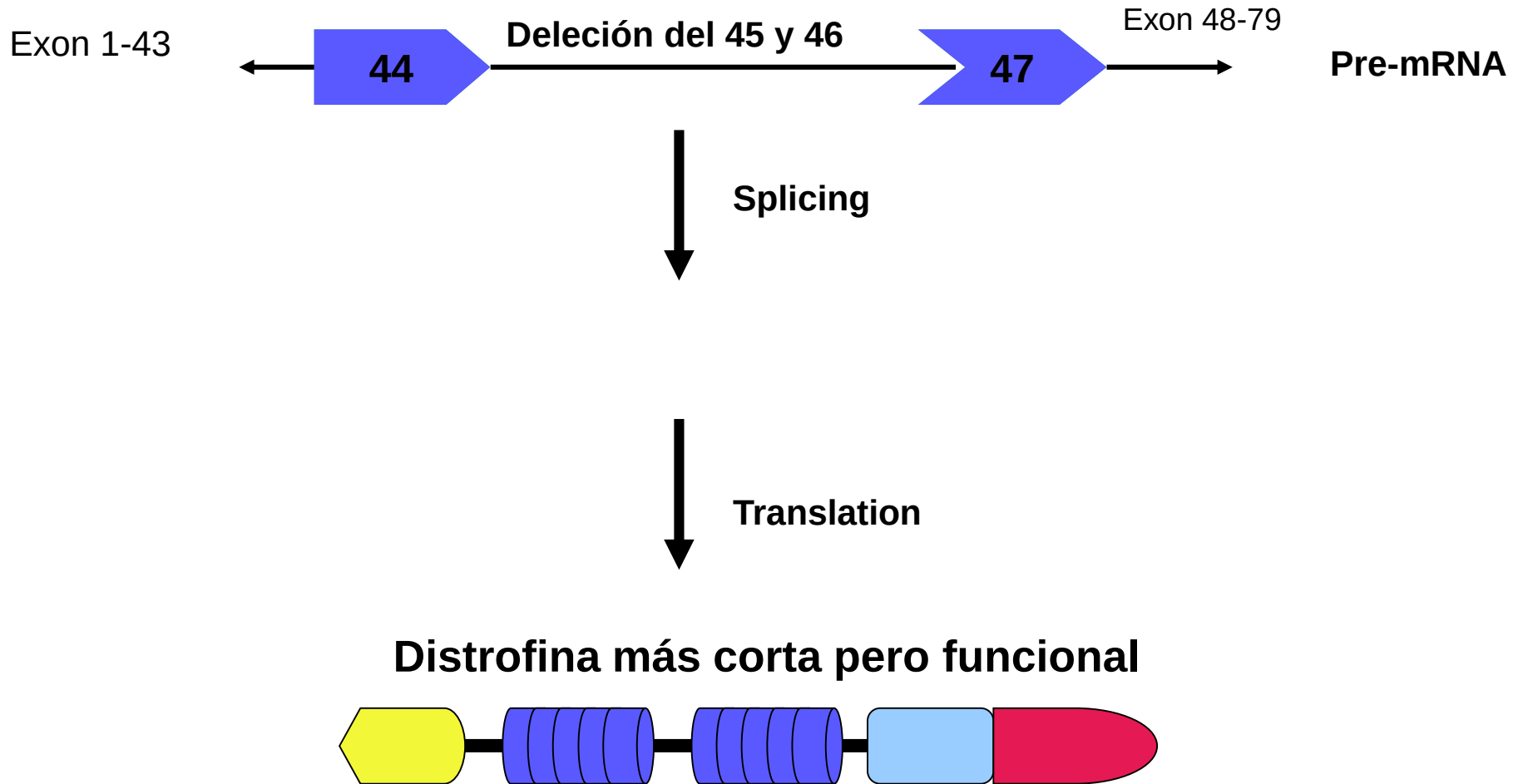


- 2,400.000 pares de bases (1% del cromosoma X, 0,1% de todo el genoma)
- 79 exones (13.973 pares de bases, 0,5% del gen)
- 8 promotores
- Expresión en músculo, corazón, cerebro, Purkinje, retina, células de Schwann

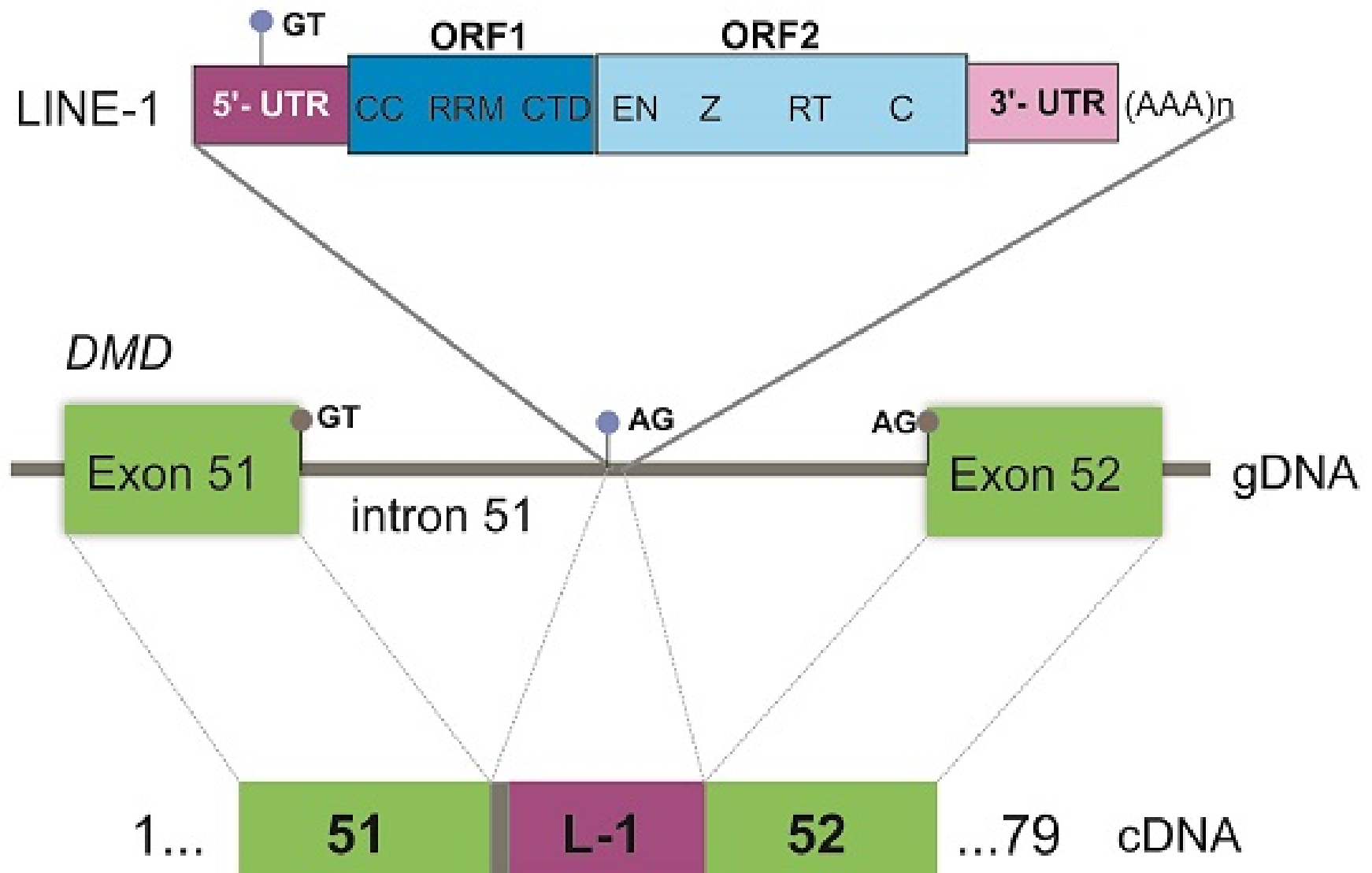
Rotura del marco de lectura en DMD



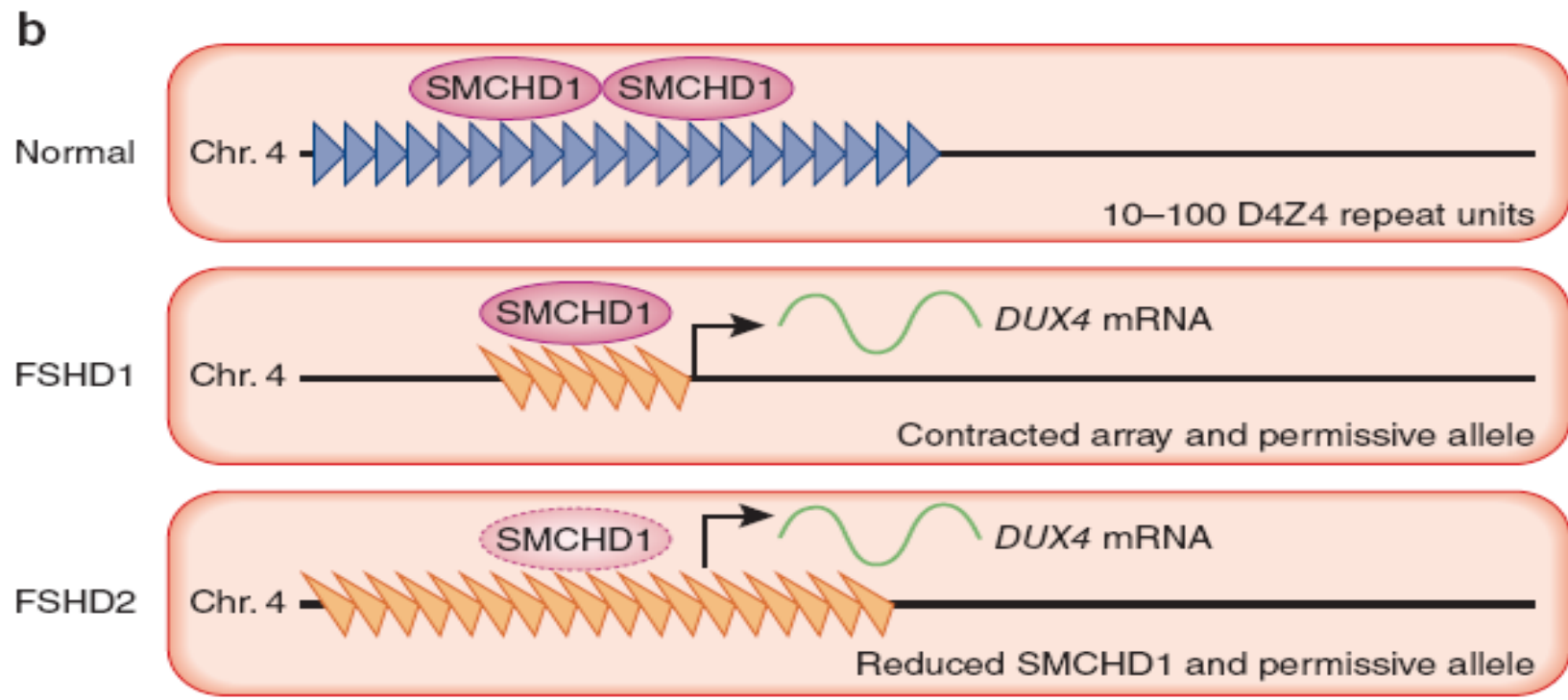
Mantenimiento del marco de lectura de en DMB



NEOEXON CREADO POR UNA MUTACION INTRONICA



**DISTROFIA
FACIOESCAPULOHUMERAL
TIPO 1 y 2**



Genética FSH

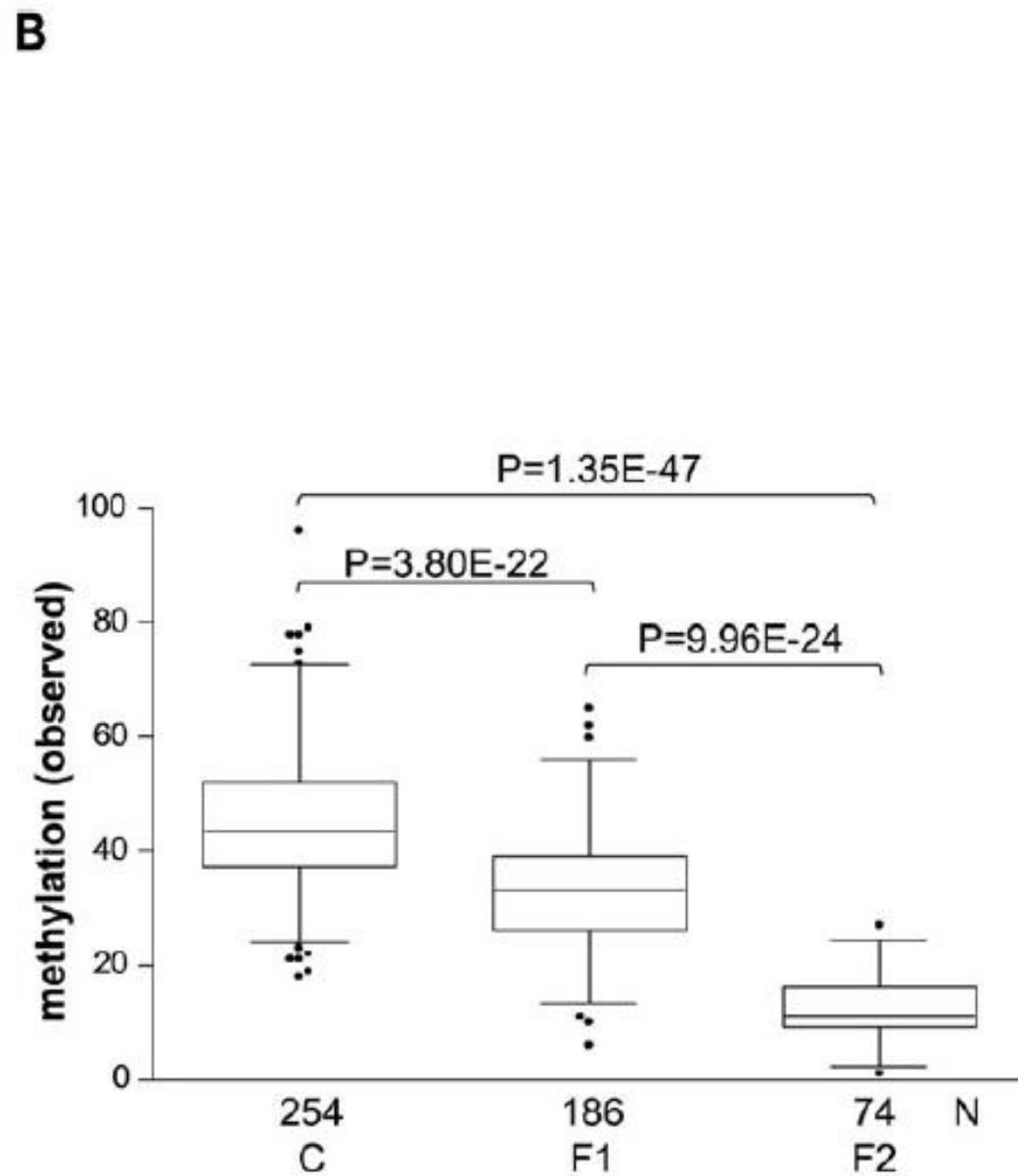
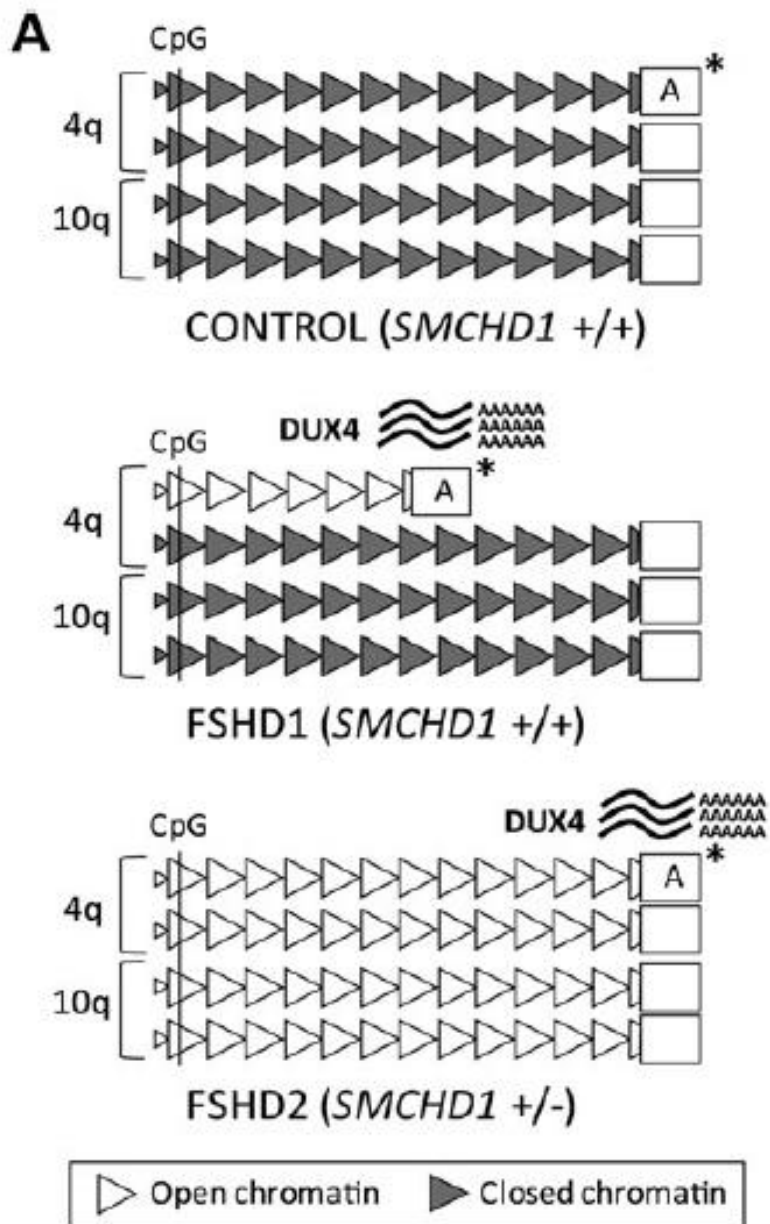
4q and 10q alleles in controls

Haplotype	SSLP sequence				SSLP <i>n</i>	D4Z4 SNP (G/C)		%
	CA _{<i>n</i>} -MA-CA _{<i>n</i>}	G/T	CT _{<i>n</i>}	8 nt		G	C	
4qA161	CA ₁₀ -AA-CA ₁₀	G	CT ₆	-	171	108	-	86%
4qA163	NA				7	5	-	4%
4qA166	CA ₉ -AA-CA ₈	T	CT ₇	+	22	14	1	11%
4qB161	NA				5	4	-	2%
4qB163	CA ₁₀ -AA-CA ₁₁	G	CT ₆	-	165	107	1	68%
4qB162	CA ₁₆	T	CT ₇	+	12	-	10	5%
4qB164	NA				3	-	3	1%
4qB166	CA ₁₈	T	CT ₇	+	8	1	6	3%
4qB168	CA ₁₉	T	CT ₇	+	51	-	42	21%
10qA164	NA				16	NA		4%
10qA166	CA ₉ -AA-CA ₈	T	CT ₇	+	428	NA		96%

- Delección D4Z4
- Haplotipo 4qA161
- Hipometilación
 - ADN
 - Histonas

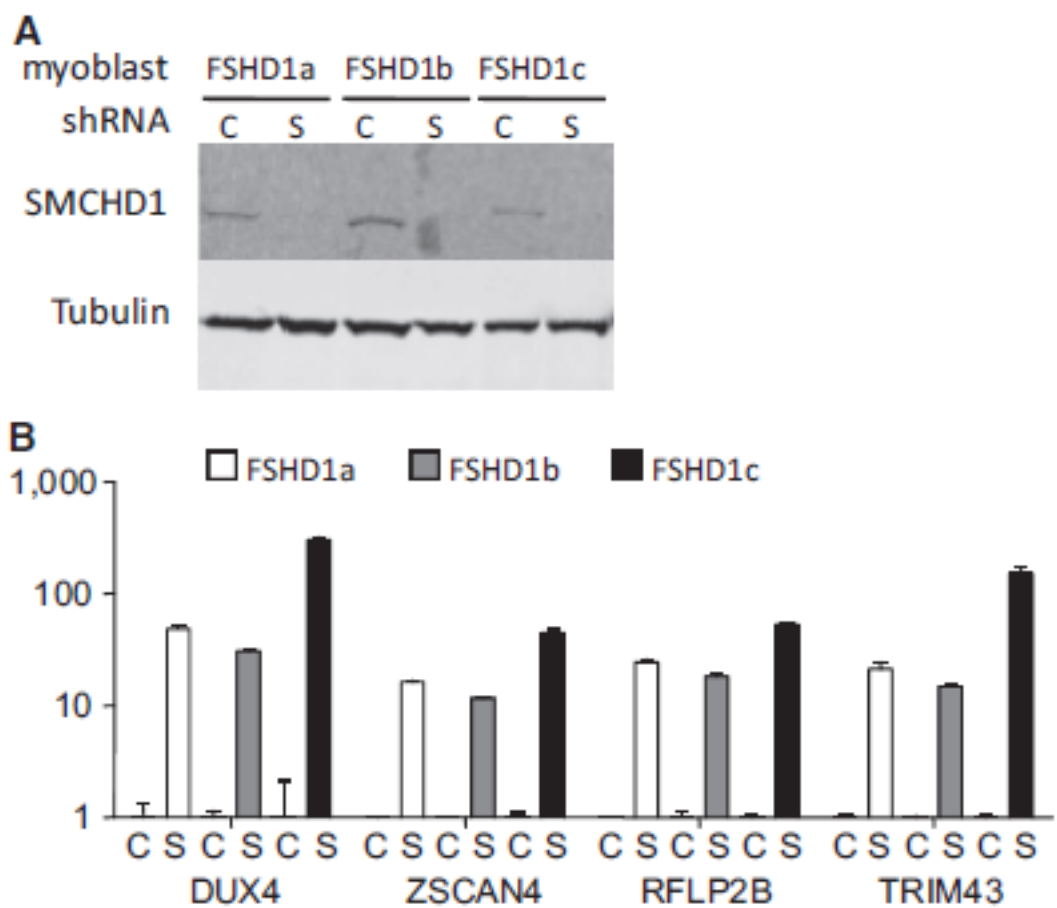
4q alleles with FSHD-sized D4Z4 repeat

Haplotype	<i>n</i>	Nonpathogenic	Pathogenic
4qA161	86	-	86
4qA166	2	2	-
4qB163	17	17	-



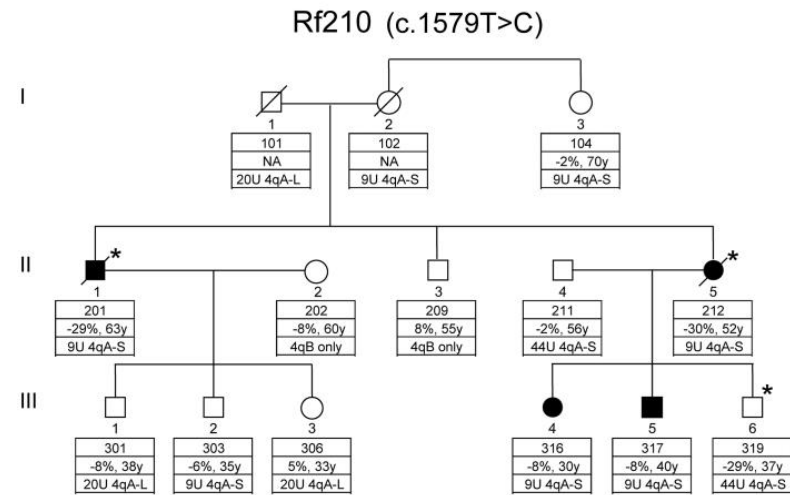
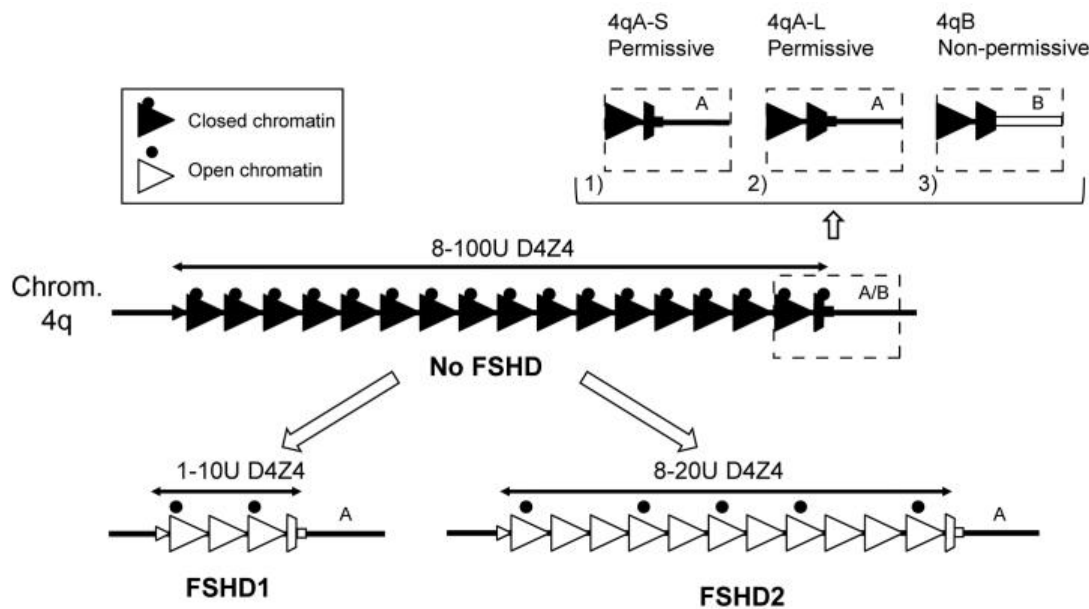
The FSHD2 Gene *SMCHD1* Is a Modifier of Disease Severity in Families Affected by FSHD1

Sabrina Sacconi,^{1,2,*} Richard J.L.F. Lemmers,³ Judit Balog,³ Patrick J. van der Vliet,³ Pauline Lahaut,¹ Merlijn P. van Nieuwenhuizen,⁴ Kirsten R. Straasheijm,³ Rashmie D. Debipersad,³ Marianne Vos-Versteeg,⁴ Leonardo Salviati,^{5,6} Alberto Casarin,^{5,6} Elena Pegoraro,⁷ Rabi Tawil,⁸ Egbert Bakker,⁴ Stephen J. Tapscott,⁹ Claude Desnuelle,^{1,2,10} and Silvère M. van der Maarel^{3,10}

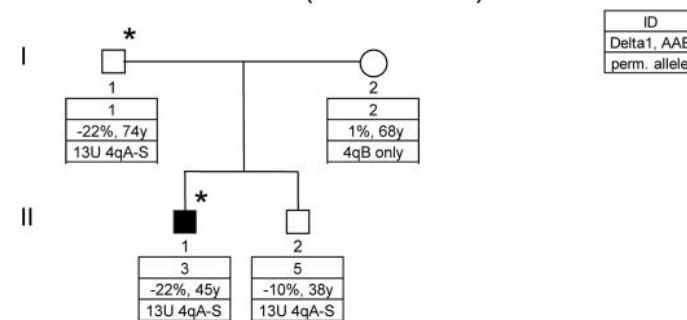


Mutations in *DNMT3B* Modify Epigenetic Repression of the D4Z4 Repeat and the Penetrance of Facioscapulohumeral Dystrophy

The American Journal of Human Genetics 98, 1020–1029, May 5, 2016



Rf732 (c.2072C>T)



Family	Nr	Coriell ID	Gender	DNMT3B mutation	Metaphases	Anomalies	Details anomalies
Rf210	316	-	F	NM_006892.3	100 (2013)	0	
Rf210	317	-	M	WT	100 (2015)	0	
Rf210	319	-	M	c.[1579T>C];[=]	100 (2013)	3	One cell with a multiradial of chromosome 1 (p,p,q,q,q) and a triradial of chromosome 16 (p,q,q) (see B-1) Two cells with decondensation (stretching) in the pericentromeric region of chromosome 1 (see B-2)
Rf732	3	-	M	c.[2072C>T];[=]	100 (2015)	4	One cell with a triradial of chromosome 16 (p,q,q) One cell with a triradial of chromosome 16 (p,q,q) and decondensation in the pericentromeric region of chromosome 1 (see B-3) One cell with a deletion of 9q (see B-4) One cell with a deletion of 16q
Rf1178	1	GM08729	M	WT	95	0	See ref. 32 and 35
Rf1178	2	GM08714	M	c.[1807G>A];[2421-11G>A]	28	2	One cell with a deletion of 1q and one cell with an extra 1q. See also ref. 32 and 43
Rf1178	3	GM08728	F	c.[1807G>A];[=]	61	0	See ref. 32 and 35

MITOCHIP

- Elegir el tejido (músculo, mucosas, orina)
- Tener en cuenta la heteroplasmia
- Algunas mutaciones asocian un fenotipo
- Los estudios bioquímicos pueden ser inespecificos

MITOCHIP

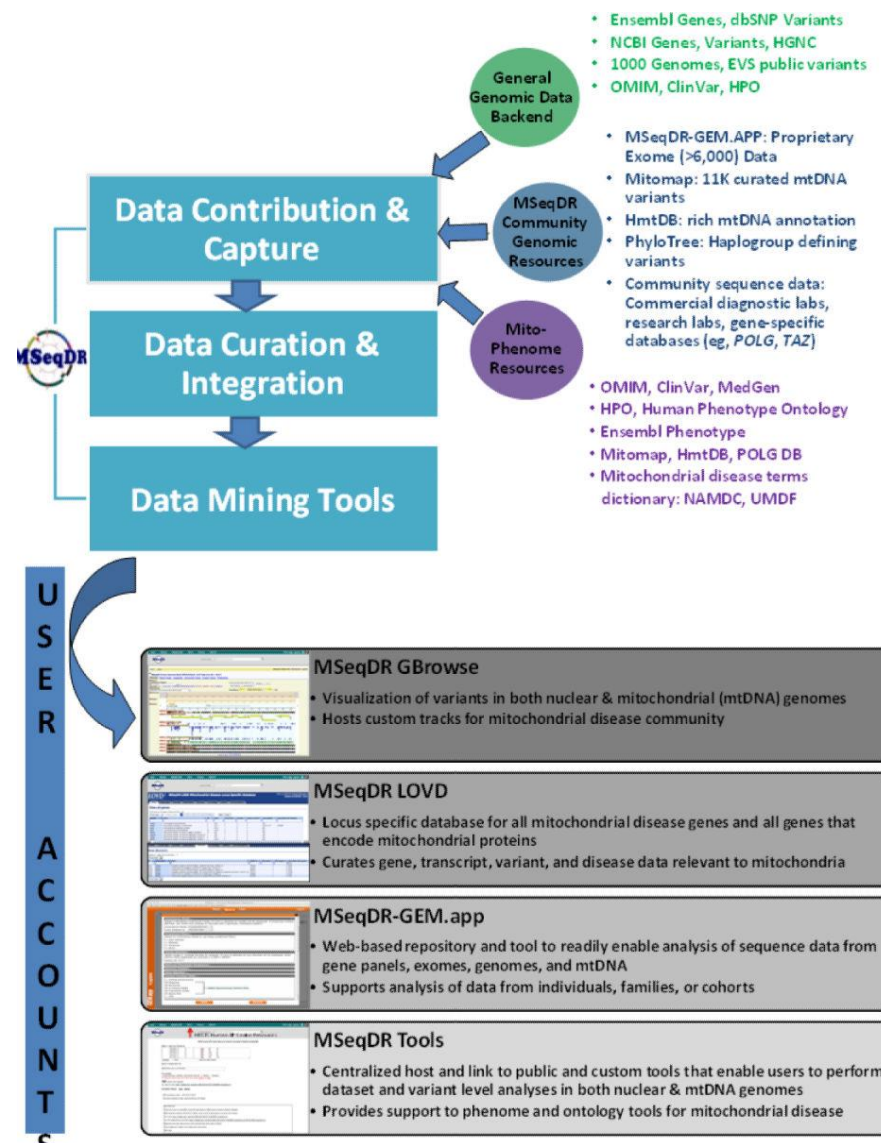
Comparison of sequencing costs per nucleotide for MitoChip assay versus two-step dye terminator sequencing (NIST, Gaithersburg, MD)

MitoChip Assay (n = 1 sample)		Dye Terminator Sequencing (using two-step PCR with M13 tagged primers) (n = 1 sample)	
Cost of 1 MitoChip \$400	=	Primary and secondary PCR, clean up, quantification = \$160	
Cost of PCR, labeling, hybridization reagents/chip = \$50		DNA sequencing (ABI3100, incl. reagent costs) = \$200	
Total cost per chip \$450	=	Total cost per sample \$360	=
Nucleotides sequenced/chip 15,400	~	Nucleotides sequenced per sample 16,500	~
Cost per nucleotide sequenced 2.9 ct	=	Cost per nucleotide sequenced 2.2 ct	=

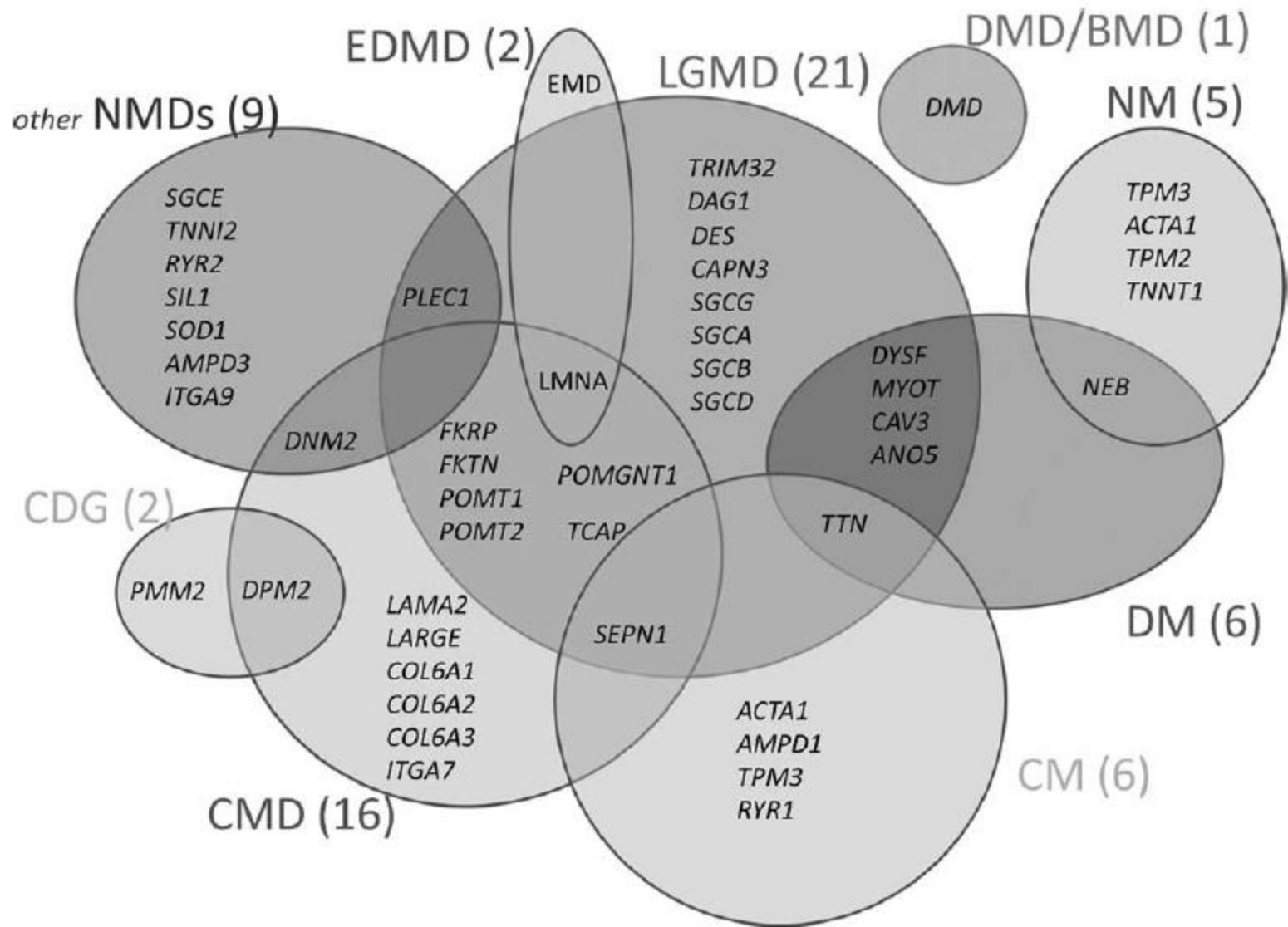
Mitochondrial Disease Sequence Data Resource (MSeqDR)

HIGHLIGHTS

- MSeqDR is a genomic data repository and analysis resource for mitochondrial disease
- MSeqDR GBrowse enhances interrogation of both genomes with custom annotation tracks
- MSeqDR LSDB curates variation in >1,300 genes relevant to mitochondrial biology
- MSeqDR GEM.app enables Web-based exome or genome dataset curation and analysis
- MSeqDR hosts bioinformatics & phenotyping tools for mitochondrial disease analyses



DISTROFIAS DE CINTURAS: HETEROGENEIDAD GENETICA/SOLAPAMIENTO FENOTIPICO



The genetic basis of undiagnosed muscular dystrophies and myopathies: Results from 504 patients

[Neurology](#). 2016 Jul 5; 87(1): 71–76.

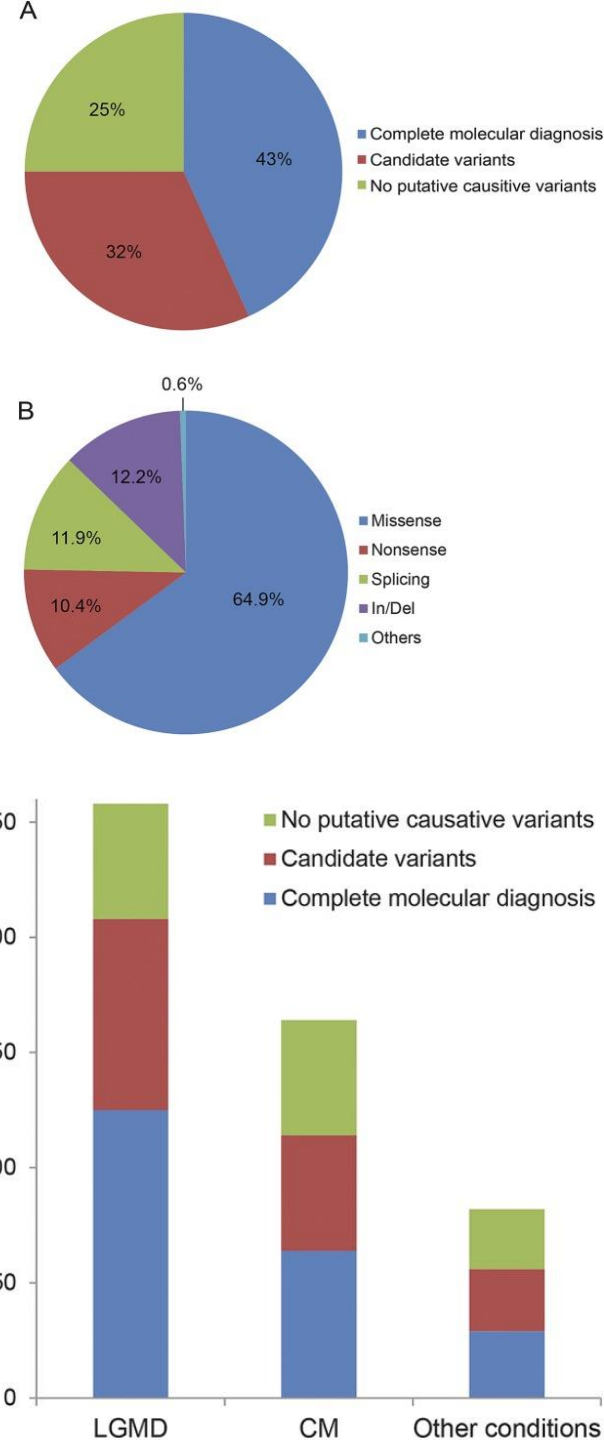
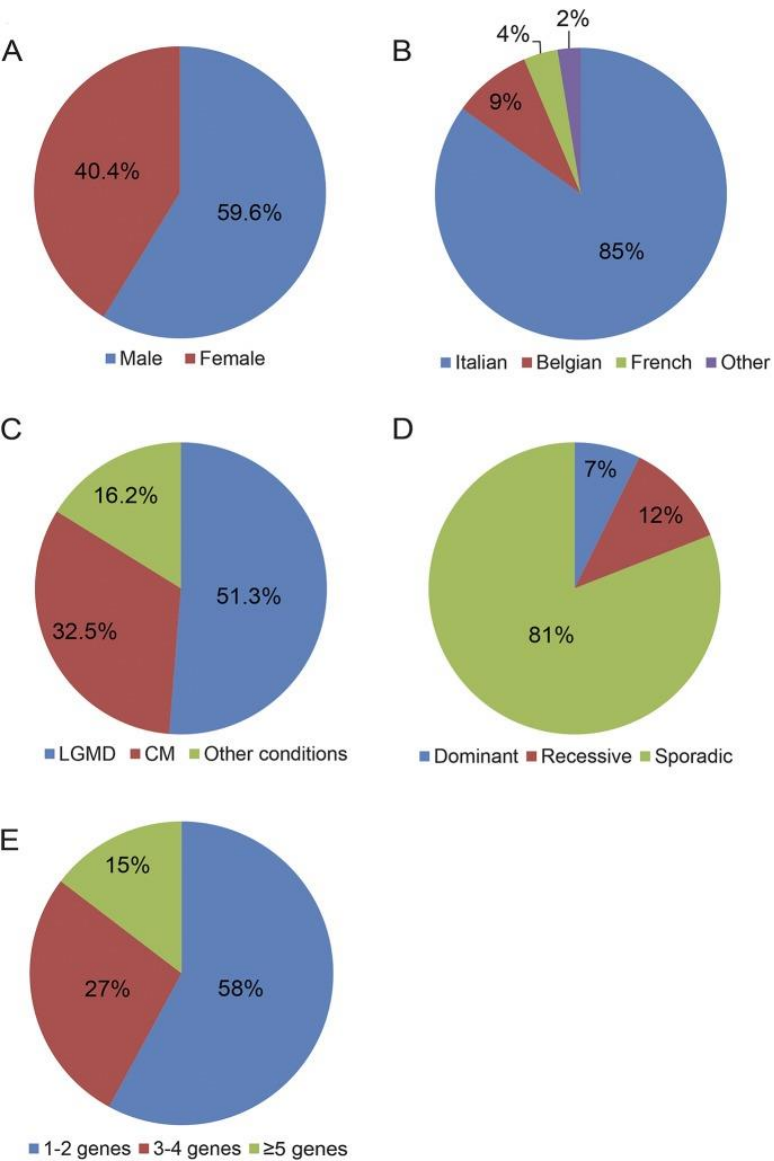
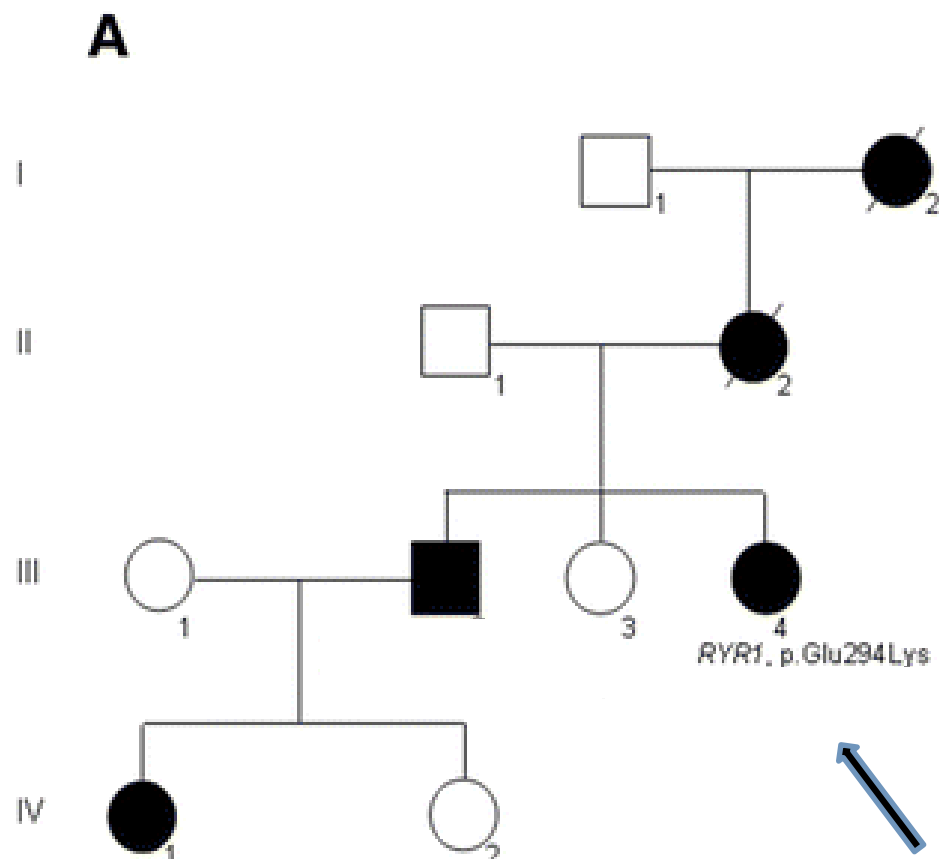


Table 1 LGMD genes ²²			
Disease	Locus	Gene	No. of patients
LGMD1B	1q22	LMNA	3
LGMD1C	3p25.3	CAV3	2
LGMD2A	15q15	CAPN3	22
LGMD2B	2p13.2	DYSF	15
LGMD2C	13q12	SGCG	4
LGMD2D	17q21	SGCA	10
LGMD2E	4q12	SGCB	6
LGMD2G	17q12	TCAP	1
LGMD2H	9q33.1	TRIM32	1
LGMD2I	19q13.3	FKRP	7
LGMD2J	2q24.3	TTN	5
LGMD2K	9q34.1	POMT1	1
LGMD2L	11p13	ANO5	15
LGMD2M	9q31	FKTN	2
LGMD2N	14q24	POMT2	6
LGMD2R	2q35	DES	1
LGMD2S	4q35.1	TRAPPC11	2
LGMD2T	3p21	GMPPB	2
LGMD2V	17q25	GAA	10

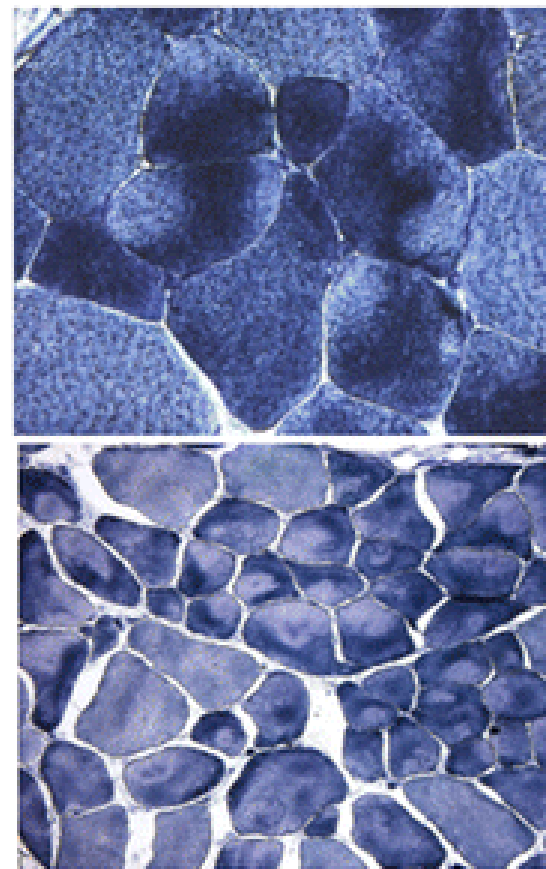
Abbreviation: LGMD = limb-girdle muscular dystrophy.

Table 2 Other myopathy genes		
Locus	Gene	No. of patients
1q42.13	ACTA1	2
1p21	AGL	2
21q22.3	COL6A2	4
2q37	COL6A3	1
11q22.3-q23.1	CRYAB	1
Xp21.2	DMD	7
19p13.2	DNM2	5
Xq28	EMD	1
7q32	FLNC	4
17q25.2-q25.3	GLA	1
3p12	GNE	3
3p22.1	GTDC2	1
3q24	GYG1	1
12q13.2	ITGA7	2
6q22-q23	LAMA2	8
Xq28	MTM1	5
17p13.1	MYH2	1
14q12	MYH7	8
5q31	MYOT	1
2q23.3	NEB	9
11q12-q13.2	PYGM	3
20p13	RYR1	25
1p36.13	SEPN1	2
18p11.32	SMCHD1	1
6q25	SYNE1	1
14q23.2	SYNE2	1
9p13	TPM2	1
1q21.2	TPM3	2

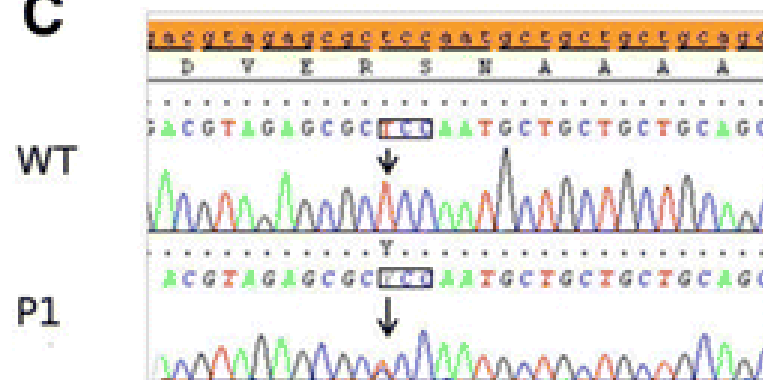
UN CASO PROBLEMA..... APARENTEMENTE FÁCIL

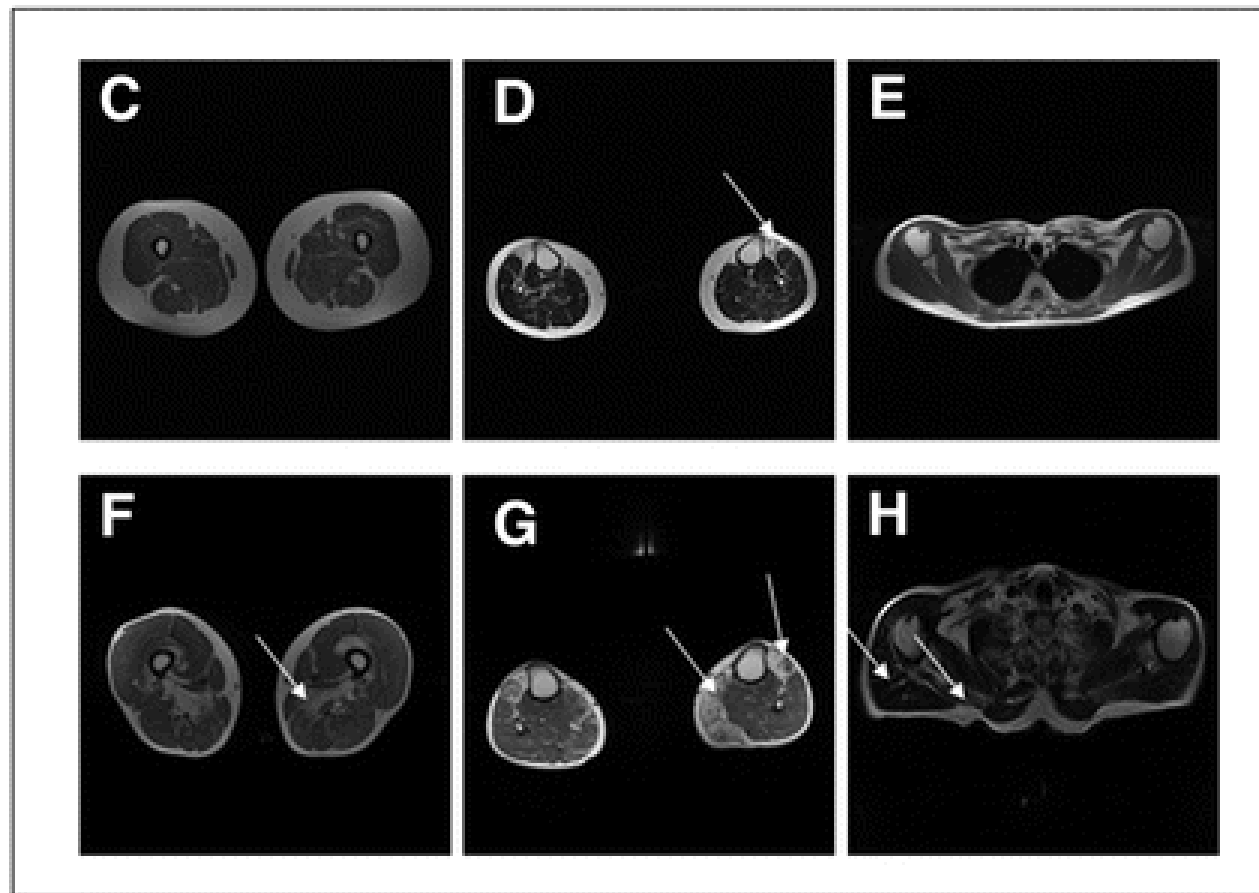
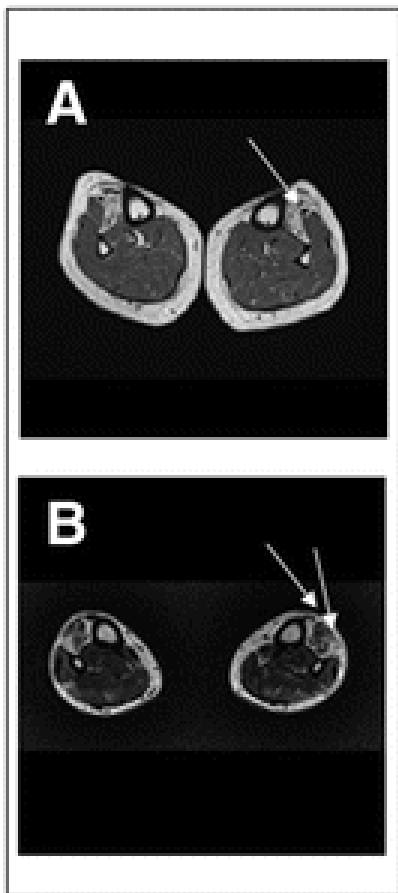


B



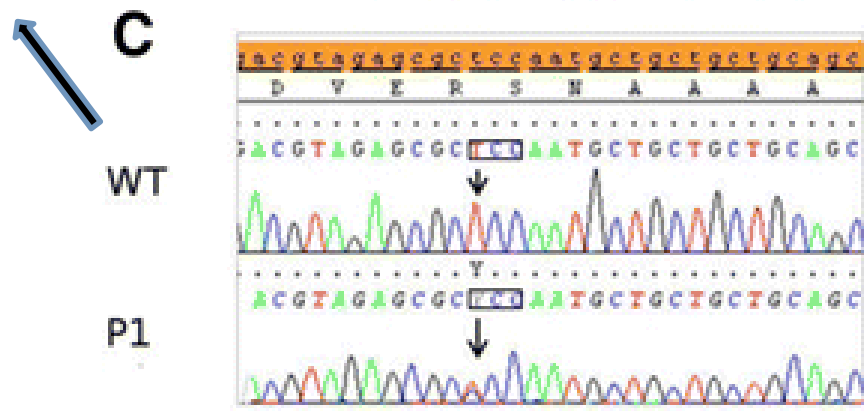
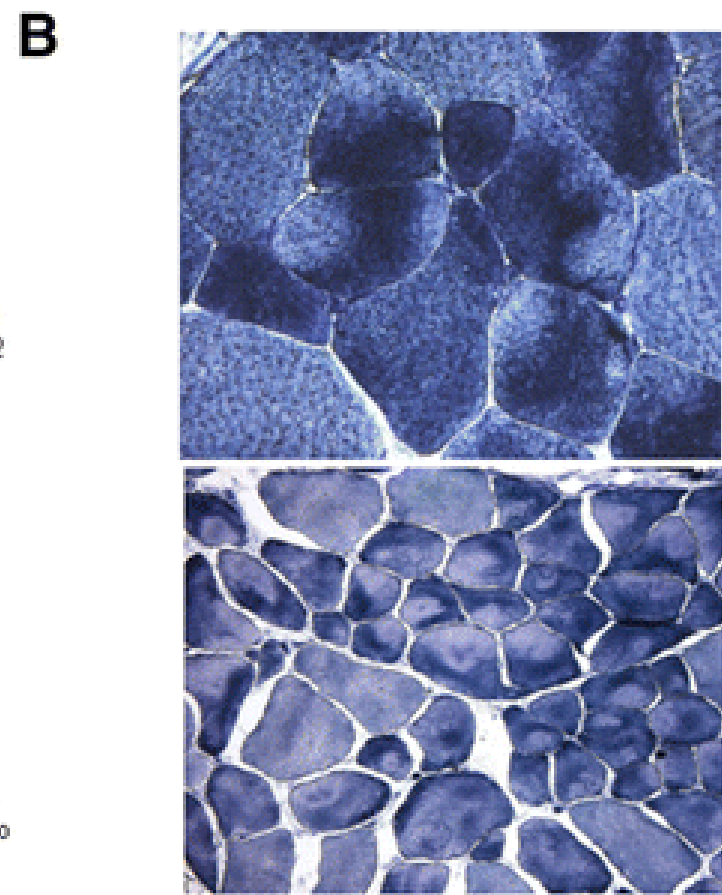
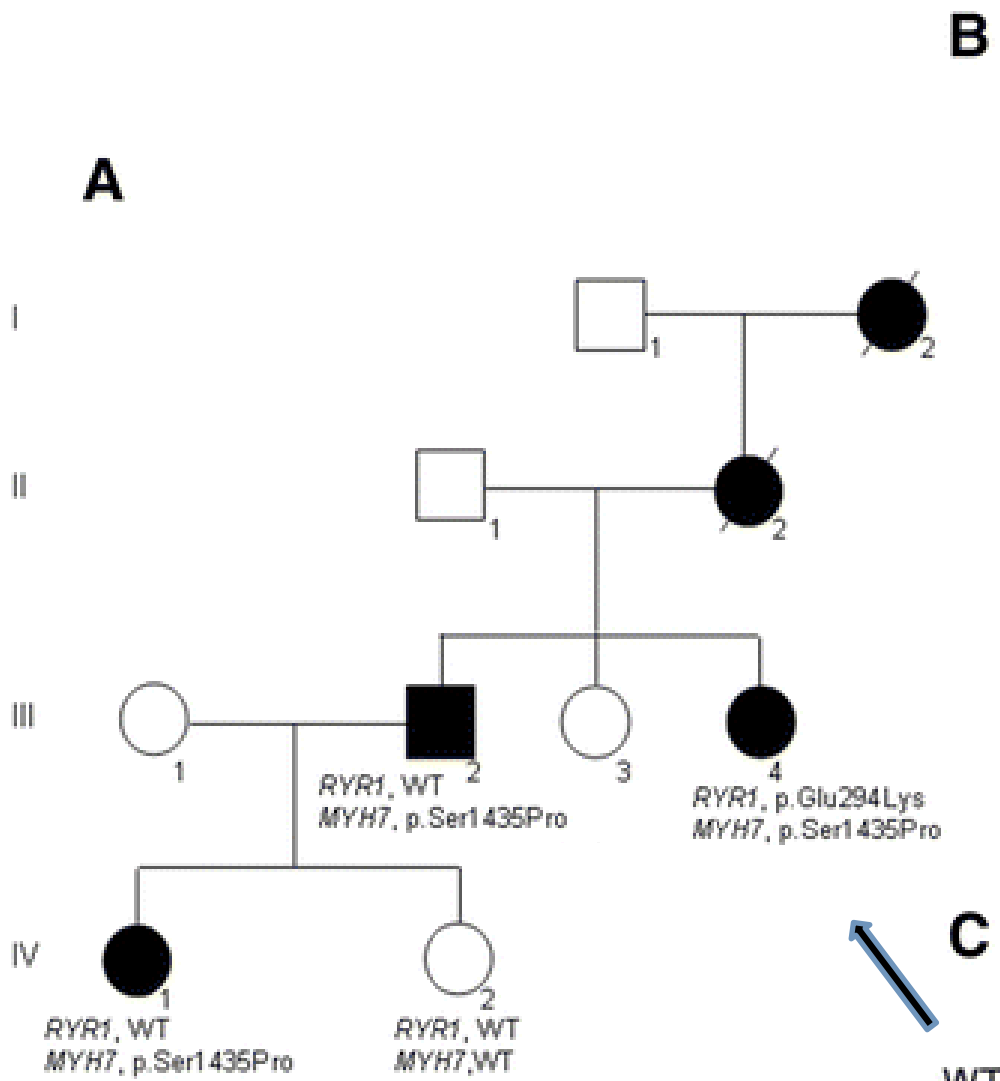
C





RM de los casos III.4 (**a**) y III.2 (**b**). Afectación selectiva de tibial anterior y en III.2, del extensor común de los dedos respetando gastrocnemio y sóleo. Seguimiento a los 3 años en III.4 (**c–e**) y III.2 (**f–h**). La imagen de III.4 esta estable pero III.2 muestra afectación de aductor y vasto intermedio en el muslo y (**f**) gemelo medio en pantorrilla (**g**). En cintura escapular se observa afectación de deltoides y esternocleidomastoideo

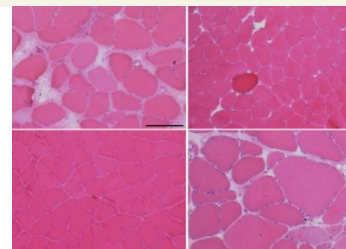
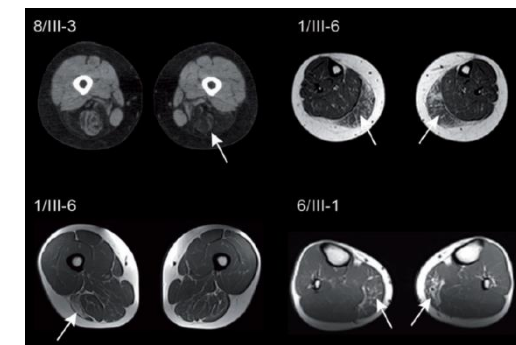
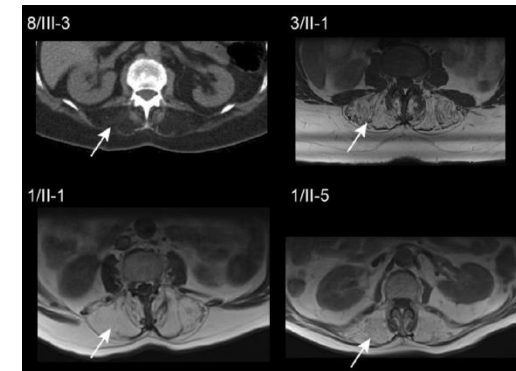
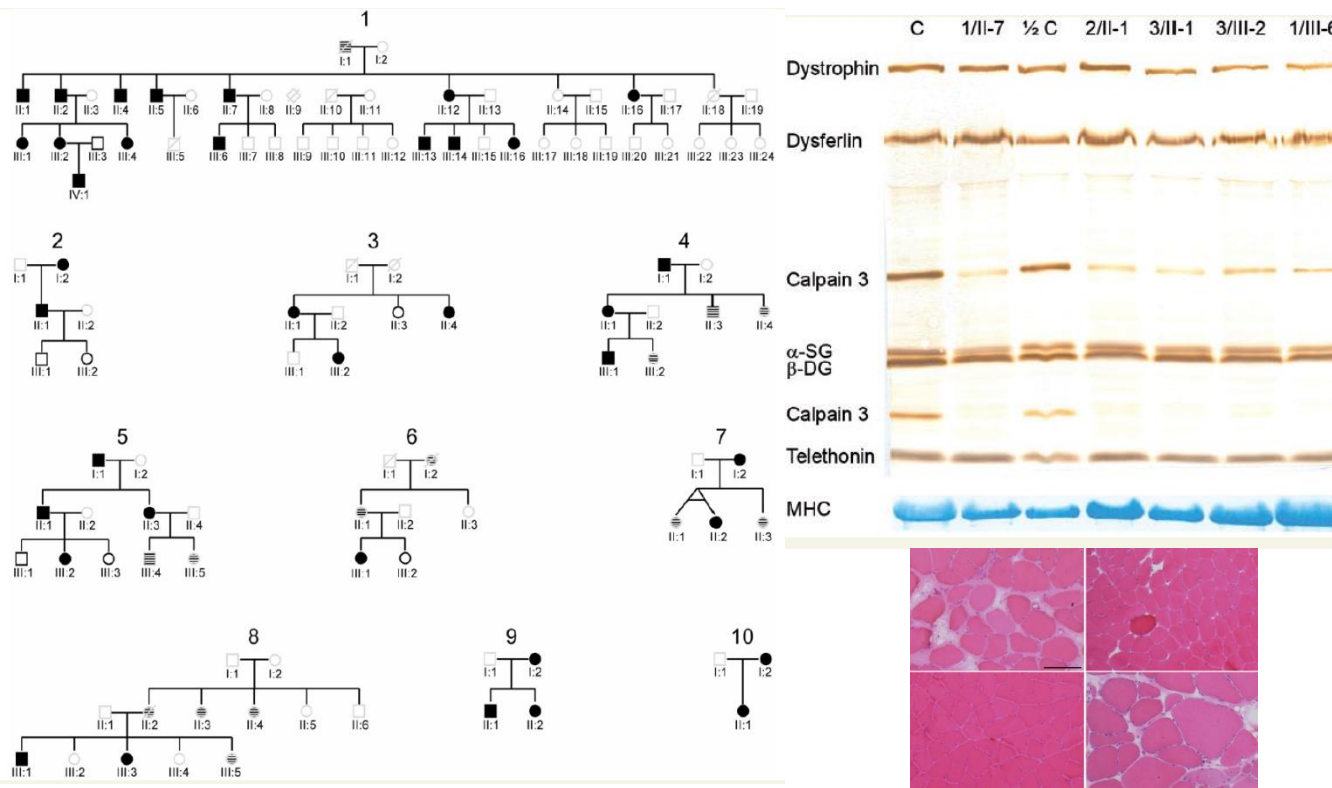
UN CASO PROBLEMA..... APARENTEMENTE FÁCIL



A heterozygous 21-bp deletion in *CAPN3* causes dominantly inherited limb girdle muscular dystrophy

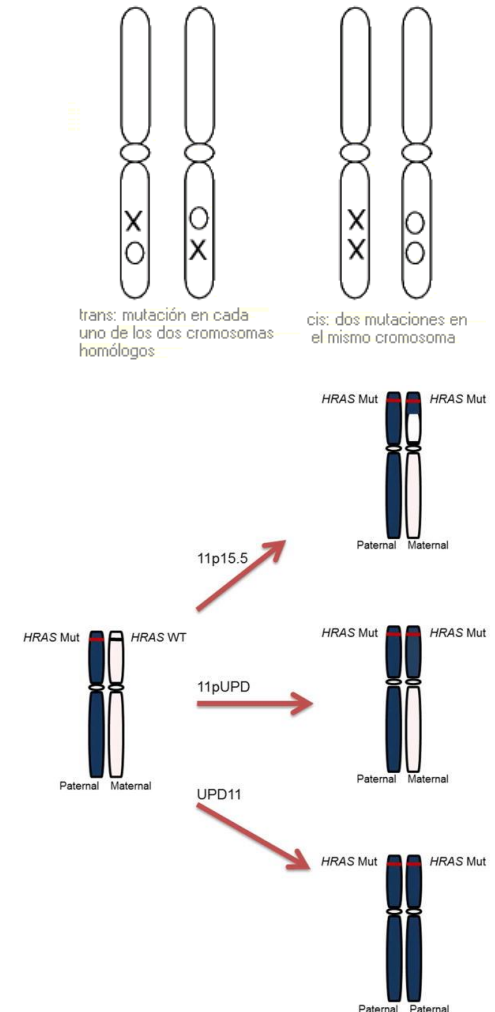
John Vissing,¹ Rita Barresi,² Nanna Witting,¹ Marijke Van Ghelue,³ Lise Gammelgaard,⁴ Laurence A. Bindoff,⁵ Volker Straub,² Hanns Lochmüller,² Judith Hudson,⁶ Christoph M. Wahl,⁷ Snjolaug Arnardottir,⁸ Kathe Dahlbom,⁹ Christoffer Jonsrud³ and Morten Duno¹⁰

A single heterozygous in-frame c.643_663del21 pathogenic variant in *CAPN3* results in a dominantly inherited form of calpainopathy. This variant has been previously identified in compound heterozygosity with other pathogenic variants in *CAPN3*, including c.2362_2363delinsTCATCT, c.550delA, p.Ala45Thr, and p.Asp419Gly (*Richard et al., 1997, Groen et al., 2007*).



ASPECTOS ÉTICOS

- Estudios de segregación
 - Confirmar si las mutaciones están en cis-trans
 - Descartar falsa paternidad o raros fenomenos como la disomía uniparental
- Hallazgos inesperados
 - Relevantes para el paciente
 - Relevantes para la sociedad



ALGUNAS CONCLUSIONES A MODO DE RESUMEN

- El diagnóstico genético es de confirmación, pero **el diagnóstico clínico es previo y orientador** del estudio molecular
- Existe **heterogeneidad clínica** (series alélicas) y **genética**, ambas muy presentes en el caso de enfermedades neuromusculares
- Los resultados genéticos tienen un margen de incertidumbre a veces difícil de establecer: es preciso contemplar el **diagnóstico como un proceso sólo provisionalmente acabado**
- La negatividad de un estudio genético en un gen concreto puede ser un **falso negativo** por problemas inherentes a la propia mutación (deleción, expansión) o por problemas técnicos (cobertura, errores..), **un falso positivo o una VUS**
- Las **nuevas tecnologías** proporcionan un screening masivo, rápido y barato, pero... **no son infalibles y deben ser confirmadas por métodos tradicionales (SANGER) o completadas con otras (MLPA, Southern,..)**. Su rendimiento basal en ENM complejas está en el 30%
- El diagnóstico molecular puede no ser urgente en unos casos (no a cualquier precio), pero es **imprescindible para el consejo genético, el consejo reproductivo y para investigar/aplicar posibles terapias**

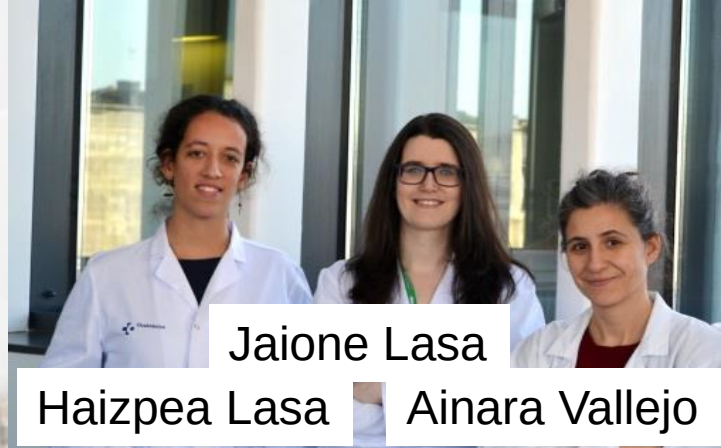


Martxel Dehesa

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Haizpea Lasa

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