Genetic Modifiers of Duchenne Muscular Dystrophy

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In relation to this presentation, the lead author has no conflict of interest to disclose.

United Dystrophinopathy Project: Prospective Genotype/Phenotype Database Participating Centers

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Washington Univ., St. Louis	A. Pestronk, A. Connolly
Children's Hospital of Philadelphia	R. Finkel
University of Minnesota, Minneapolis	J. Day
Children's Hospital of Cincinnati	B. Wong
University of California, Davis	C. McDonald

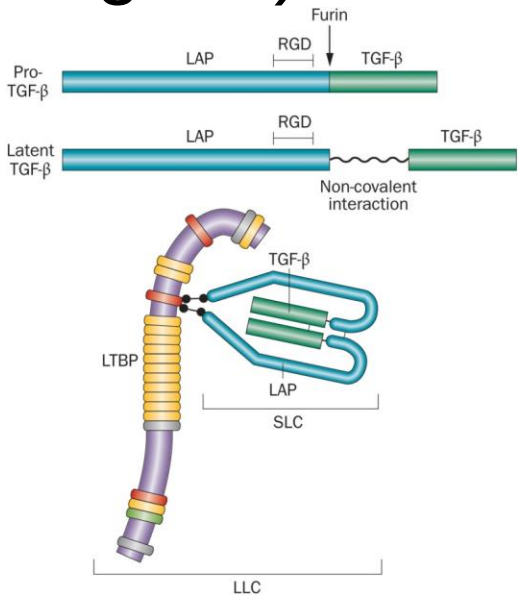
What are modifier genes of DMD and why look for them?

- "Modifiers" = A genetic interaction in which an allele from one gene (= the modifier) masks the phenotype caused by a mutation in another gene (= the dystrophin gene)
- "Validated" modifier of DMD:

Common alleles in *LTBP4*,

Latent Transforming Growth

Factor-β Binding Protein 4



Lafyatis, R. (2014) Transforming growth factor β—at the centre of systemic sclerosis *Nat. Rev. Rheumatol.* doi:10.1038/nrrheum.2014.137

Outline of the talk:

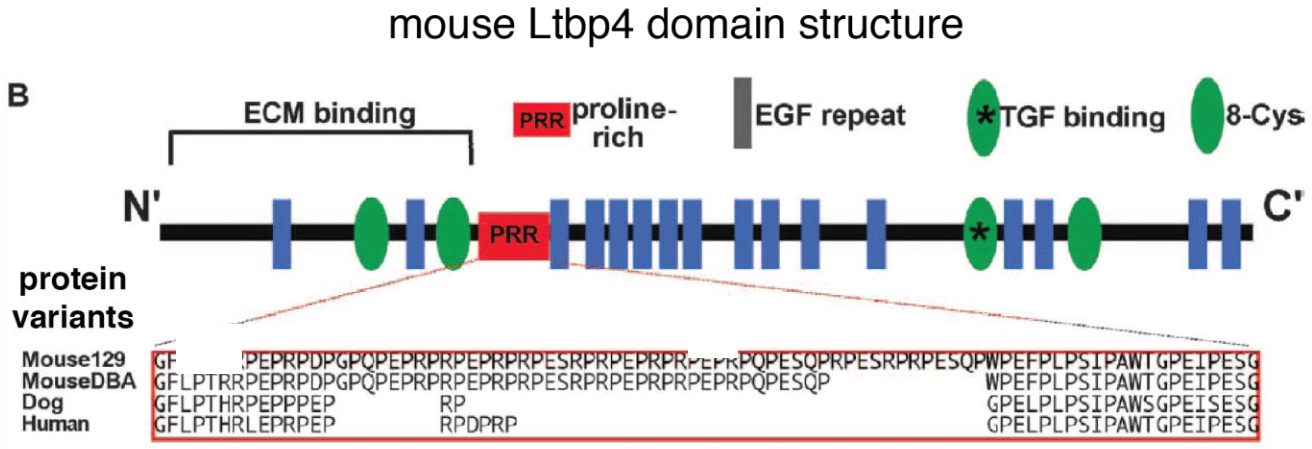
- "Modifier genes" of inherited muscular dystrophies:
  - Mouse models implicating *Ltbp4* / TGF-β signaling
  - Human *LTBP4* variants in DMD patients
  - Replication studies of additional candidate genes
- Genome-Wide Association Study (GWAS) for Loss of Ambulation: the United Dystrophinopathy Project (UDP)
  - Refining of the *LTBP4* association signal
  - SNP associations exceeding a genome-wide significance threshold (P value < 5×10^{-8}) for a DMD phenotype

Mouse *Ltbp4* modifies of muscular dystrophy

Elizabeth M McNally, MD,PhD
Northwestern University

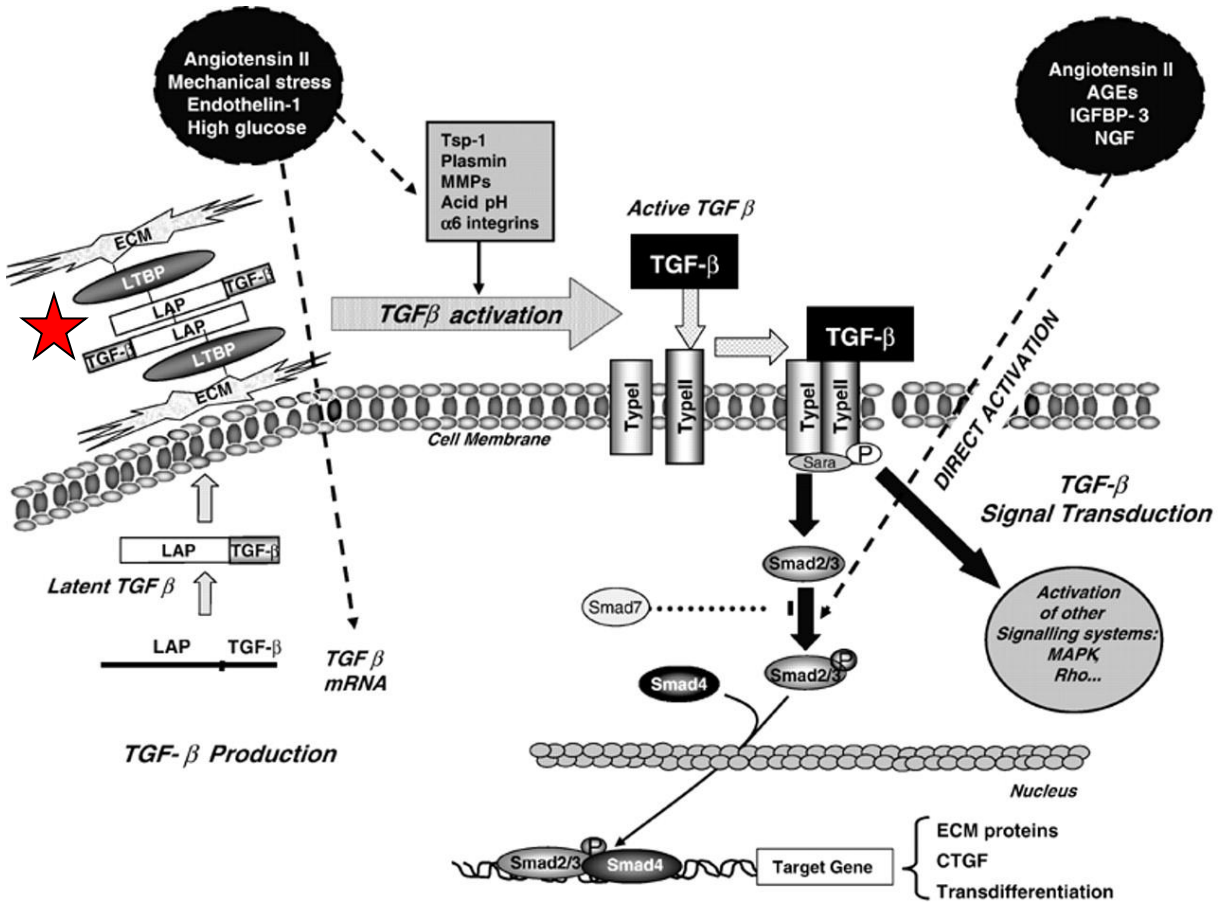
Latent TGF-beta-binding protein 4 modifies muscular dystrophy in mice.
Heydemann A, Ceco E, Lim JE, Hadhazy M, Ryder P, Moran JL, Beier DR, Palmer AA, McNally EM. J Clin Invest. 2009 Dec;119(12):3703-12.

Targeting latent TGFβ release in muscular dystrophy.
Ceco E, Bogdanovich S, Gardner B, Miller T, DeJesus A, Earley JU, Hadhazy M, Smith LR, Barton ER, Molkentin JD, McNally EM. Sci Transl Med. 2014 Oct 22;6(259):259..



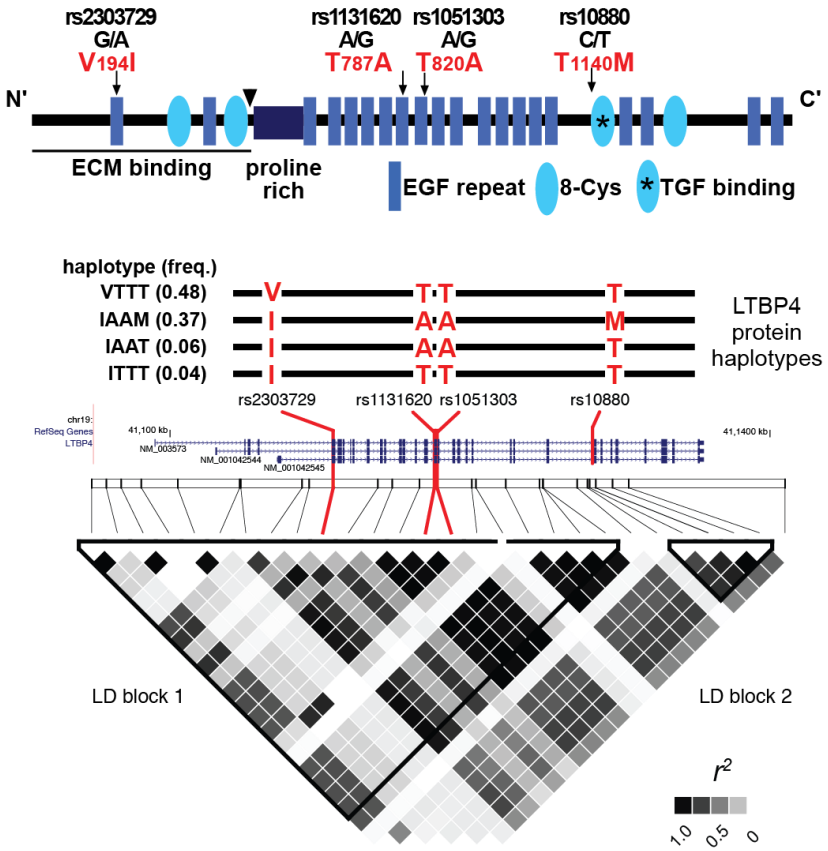
Mouse QTL for limb-based skeletal muscle damage (Evans blue dye uptake) and fibrosis (hydroxyproline content) linked to the chr7 / *Ltbp4* region

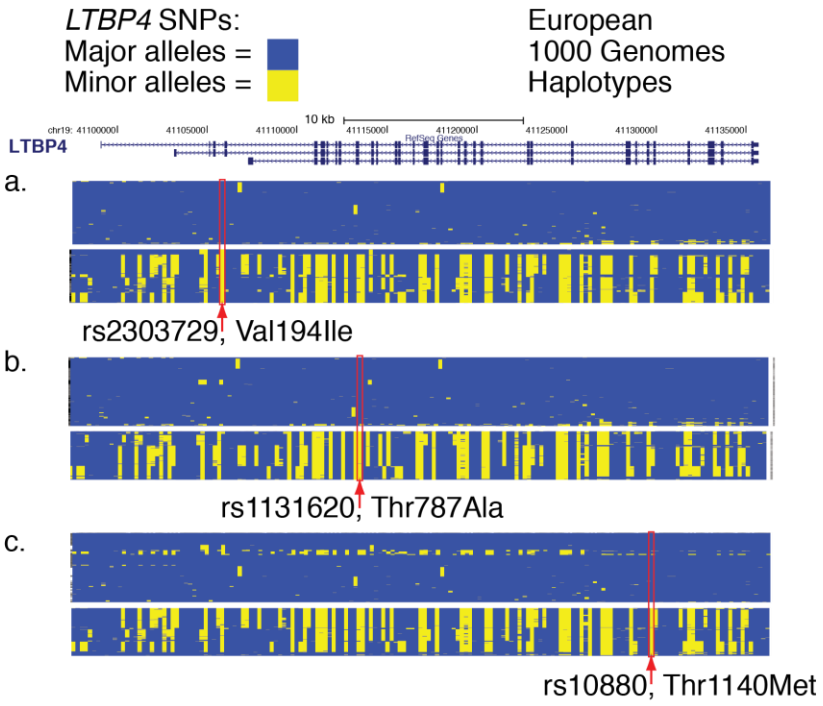
DMD modifier genes and TGF-β signaling



adapted from Marta Ruiz-Ortega et al. Cardiovasc Res 2007;74:196-206

Human *LTBP4* polymorphisms and haplotypes

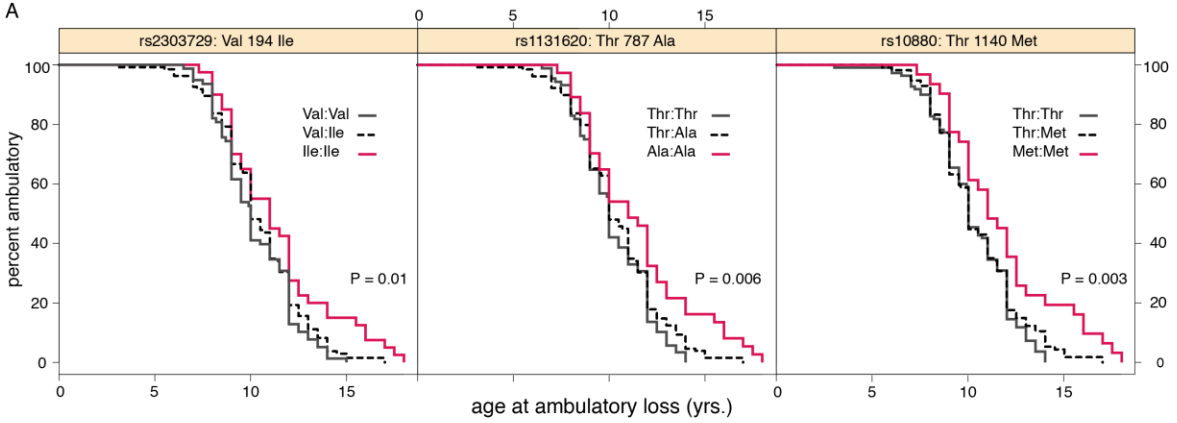




Flanigan, Kevin M., Ermelinda Ceco, Kay Marie Lamar, Yuuki Kaminoh, Diane M. Dunn, Jerry R. Mendell, Wendy M. King, et al. 2013. “LTBP4 Genotype Predicts Age of Ambulatory Loss in Duchenne Muscular Dystrophy.” *Annals of Neurology* 73 (4): 481–88

Human *LTBP4* genotypes are associated with age of ambulatory loss in DMD patients

***LTBP4* genotypes are associated with age at loss of ambulation in patients (n = 254) with Duchenne muscular dystrophy**



Flanigan et al: *LTBP4* Genotype in DMD

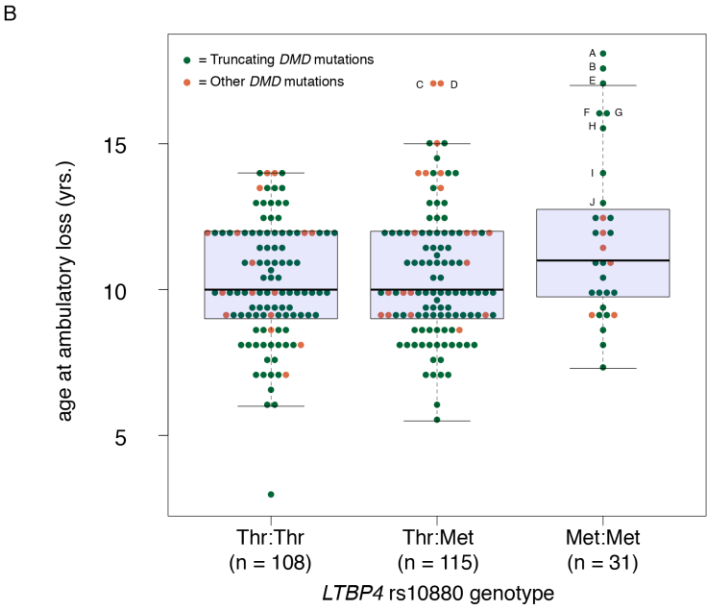


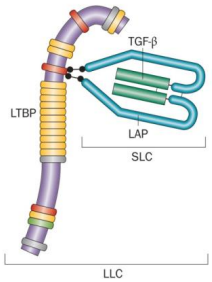
TABLE : Haplotype Analysis of Nonsynonymous *LTBP4* Variants Associated with Age of Ambulatory Loss for Duchenne Muscular Dystrophy Patients

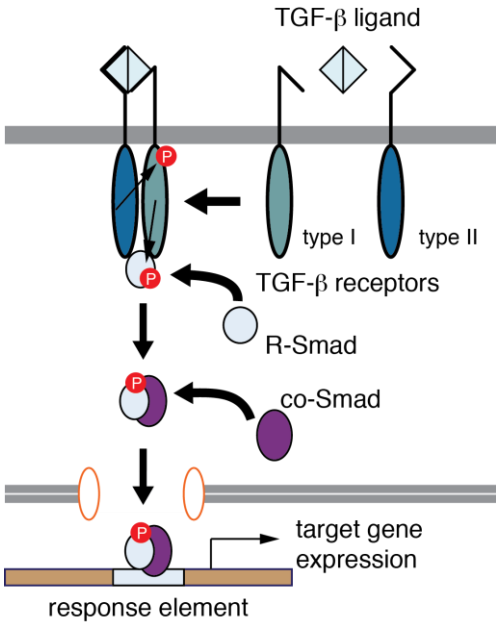
Haplotype[a]	All, n = 254, Global p^b = 0.002			Steroid Treated, n = 137, Global p^b = 0.013			Steroid Naive, n = 102, Global p^b = 0.12		
	Frequency	Score	p^c	Frequency	Score	p^c	Frequency	Score	p^c
VTTT	0.53	−1.51	0.1	0.52	−1.04	0.3	0.52	−1.12	0.3
IAAM	0.31	3.43	6×10^{-4}	0.32	2.92	0.004	0.29	1.91	0.06

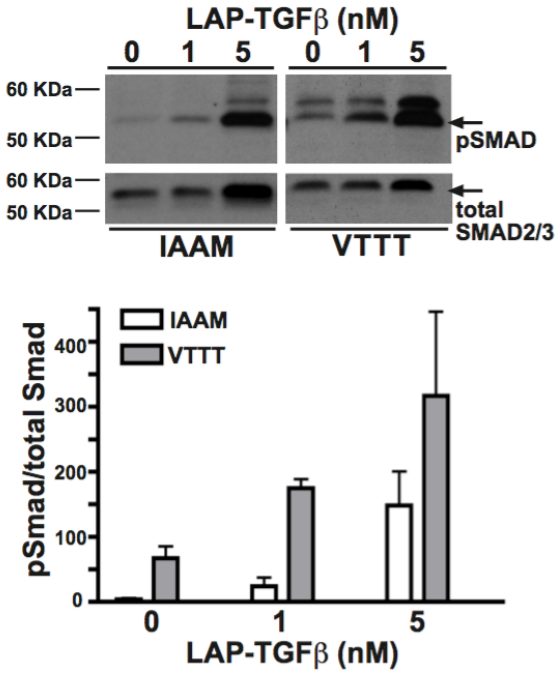
[a]These haplotypes consist of single nucleotide polymorphisms rs2303729, rs1131620, and rs10880, respectively.
[b]The haplo.stats package was used to test for association between haplotypes and age of ambulatory loss as a quantitative trait using a recessive model.
[c]Probability value from the chi-square, $df = 1$, distribution of the haplotype-specific score.

from Flanigan et al., LTBP4 genotype predicts age of ambulatory loss in Duchenne Muscular Dystrophy, Ann Neurol. 73(4):481-488 (2013).

Human *LTBP4* polymorphisms and activity in fibroblasts





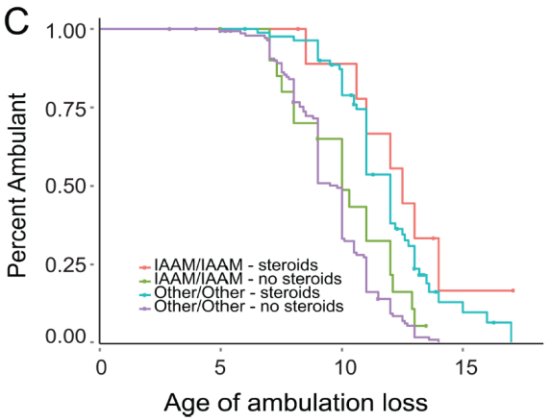


Fibroblasts homozygous for the IAAM or VTTT LTBP4 haplotypes were cultured, and latent TGFβ was added (LAP = latency associated peptide). LTBP4 protein binds and sequesters latent TGFβ, and it is predicted that IAAM binds more latent TGFβ in the matrix, leading to reduced TGFβ signaling, seen as less phosphorylated SMAD (pSMAD). IAAM fibroblasts had less pSMAD/total SMAD at baseline and at 2 different doses of latent TGFβ ($p = 0.02$, analysis of variance with repeated measures).

from Flanigan et al., LTBP4 genotype predicts age of ambulatory loss in Duchenne Muscular Dystrophy, Ann Neurol. 73(4):481-488 (2013).

LTBP4 IAAM Replication Studies

Figure 2C (from den Bergen JC, et al.) Survival plots showing the effect of the IAAM haplotype (LTBP4) in 265 patients with DMD.



European cohort (London, Ferrera, Montpelier, Leiden, Newcastle), 270 patients, P value = 0.01 (recessive): van den Bergen JC, Hiller M, Bohringer S, Vijfhuizen L, Ginjaar HB, Chaouch A, et al. Validation of genetic modifiers for Duchenne muscular dystrophy: a multicentre study assessing SPP1 and LTBP4 variants. J Neurol Neurosurg Psychiatry. 2015; 86(10):1060–5.

Figure 3B (from Flanigan et al., 2012), Survival plots showing the effect of the IAAM haplotype 238 patients from the UDP

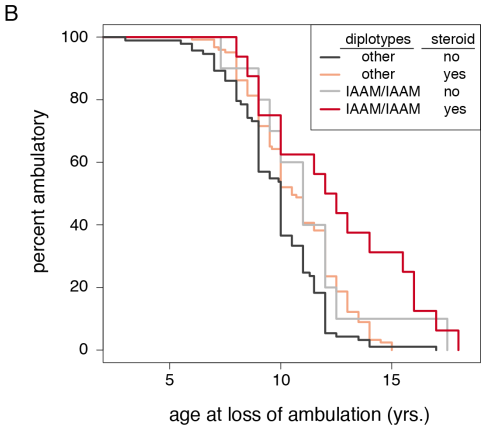
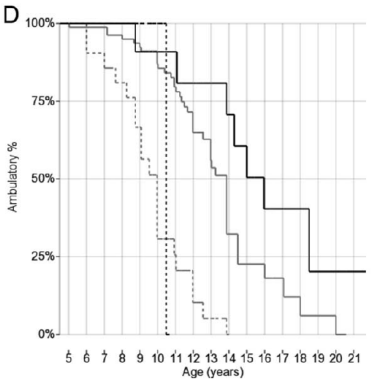


Figure 3D Caucasian CINRG cohort genotyped for LTBP4 rs10880 (n = 115), (black line = TT; gray line =CC/CT) and GC treatment (continuous lines = at least 1 year while ambulatory; dashed lines =<1 year or untreated).



CINRG cohort, 118 patients, P value = 0.02 (KM log-rank): Bello L, , et al. Genetic modifiers of ambulation in the Cooperative International Neuromuscular Research Group Duchenne Natural History Study. Ann Neurol. 2015; 77(4):684–96.

* Non-replication: Itailan cohort of 178 patients: neither SPP1 or LTBP4 associated with LoA. Barp A, Bello L et al. (2015) Genetic Modifiers of Duchenne Muscular Dystrophy and Dilated Cardiomyopathy. PLoS ONE 10(10): e0141240.

Cooperative International Research Group Duchenne Natural History Study (CINRG-DNHS): Exome-wide association study of age at LoA in a sub-cohort of (European ancestry, n = 109)

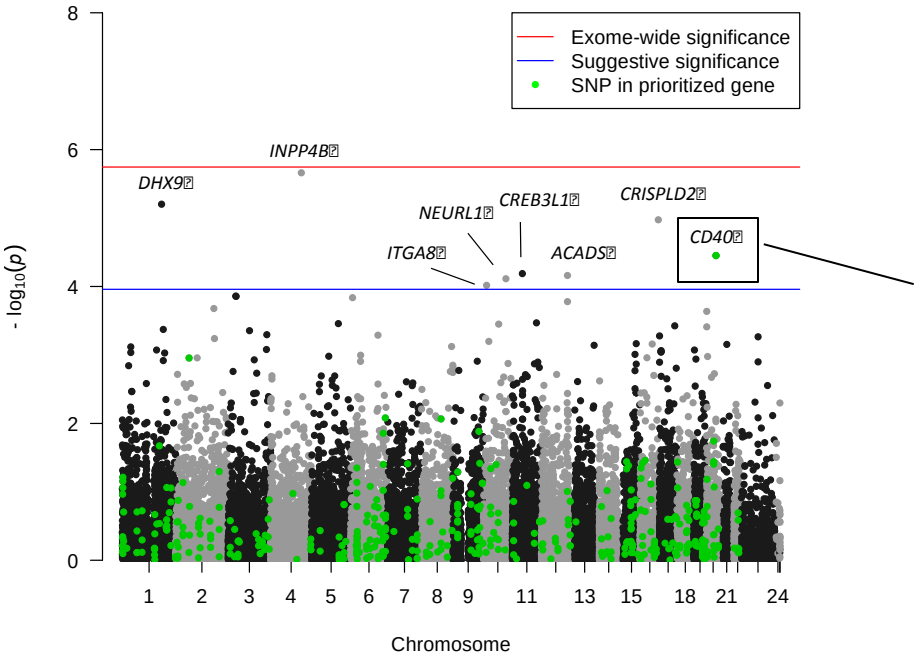
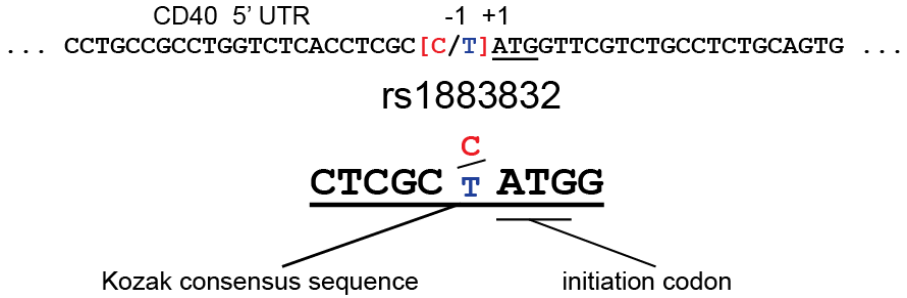


Figure 1 (from L. Bello et al. 2016) Additive genotype *P*-values of the Cox proportional hazards model with glucocorticoid treatment as a covariate are shown for 27,025 Exome Chip SNPs with MAF > 0.05. SNPs within, or < 10,000 kb upstream/downstream of prioritized genes in the NF-κB and TGFβ pathways are highlighted in green.

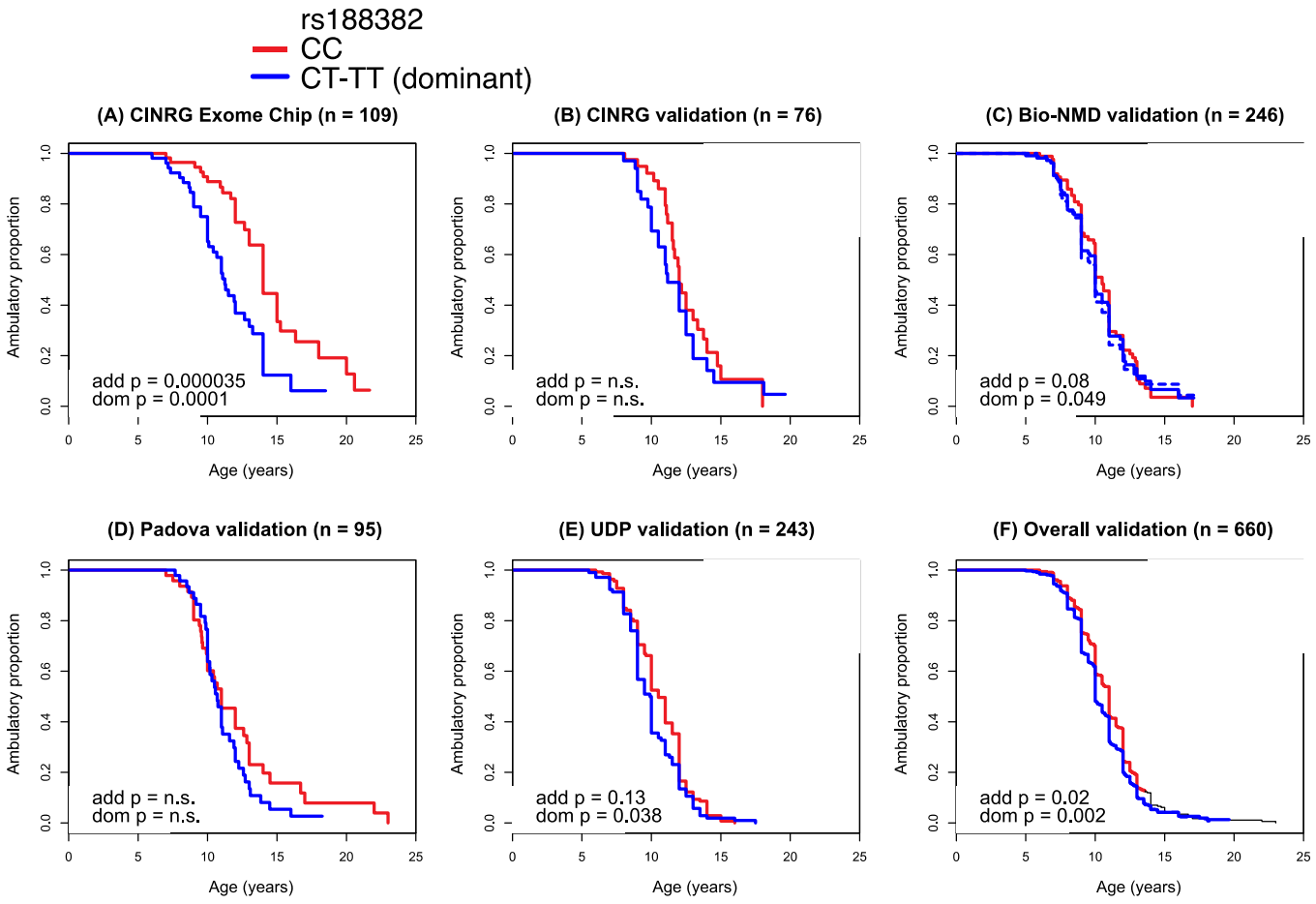
In press, AJHG: "Association study of exon variants in the NF-κB and TGFβ pathways identifies CD40 as a modifier of Duchenne muscular dystrophy", Luca Bello, Kevin M. Flanigan, Robert B. Weiss, United Dystrophinopathy Project, Pietro Spitali, Annemieke Aartsma-Rus, Francesco Muntoni, Irina Zaharieva, Alessandra Ferlini, Eugenio Mercuri, Sylvie Tuffery-Giraud, Mireille Claustres, Volker Straub, Hanns Lochmüller, Andrea Barp, Sara Vianello, Elena Pegoraro, Jaya Punetha, Heather Gordish-Dressman, Mamta Giri, Craig M. McDonald, Eric P. Hoffman, Cooperative International Neuromuscular Research Group.

CD40 is a cell surface protein and a member of the tumor necrosis factor receptor superfamily



The major allele at *CD40* rs1883832 (**C**) is associated with increased risk for Graves' disease and rheumatoid arthritis and results in increased CD40 expression, predicted to enhance a pro-inflammatory environment/response. While, the rs1883832 minor allele (**T**) is a risk allele for multiple sclerosis and is associated with reduced CD40 expression.

CD40 rs1883832 replication

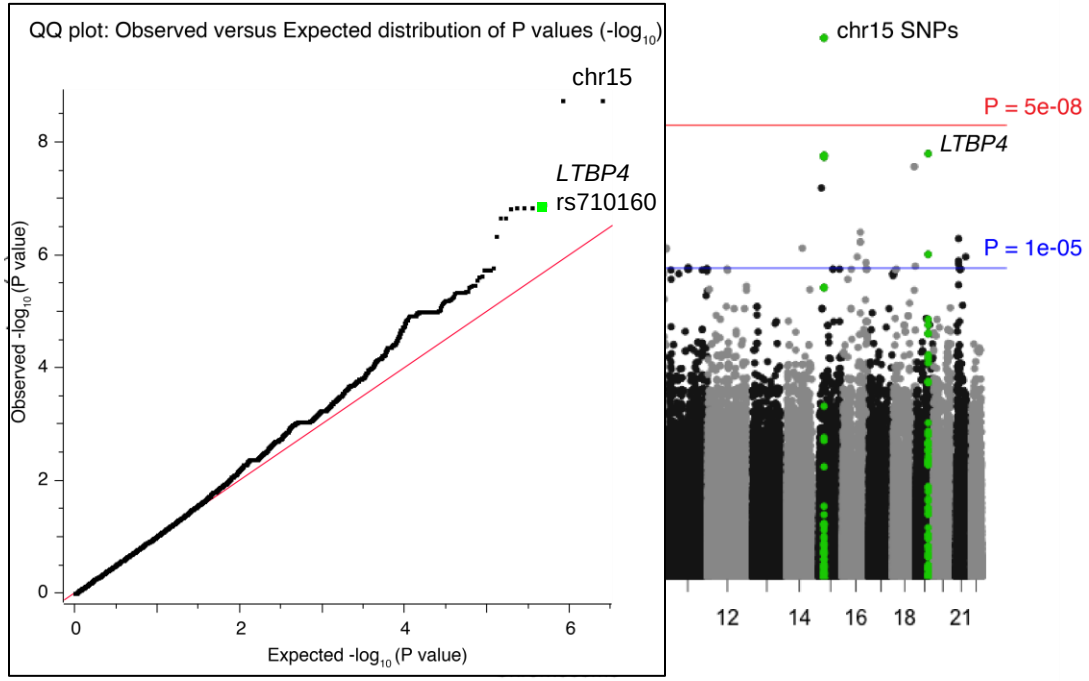


CD40 is a TNFR superfamily member and stimulation of CD40 by CD40L results in activation of MAPKs and NF-κB signaling.

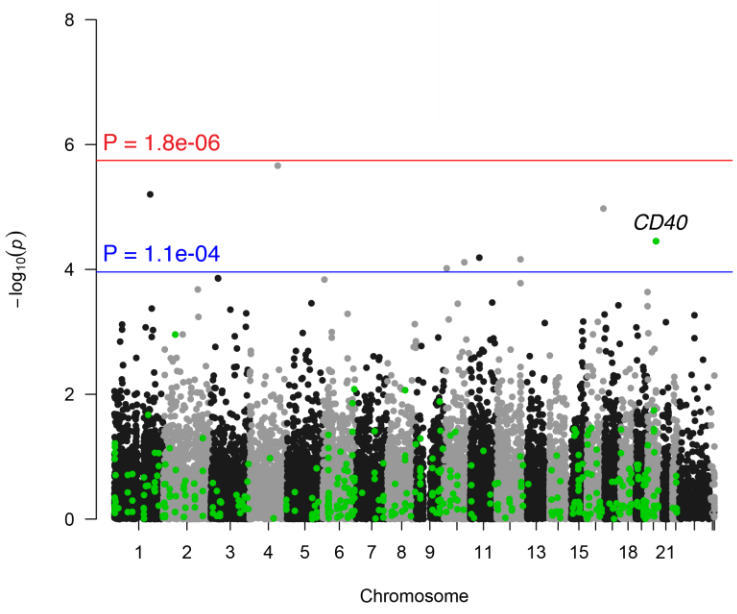
Reduced CD40-mediated cell-cell signaling in carriers of the minor rs1883832 allele might precipitate failed regeneration and fibrosis in DMD skeletal muscle.

Genome-wide Association Study of LoA in the United Dystrophinopathy Project cohort

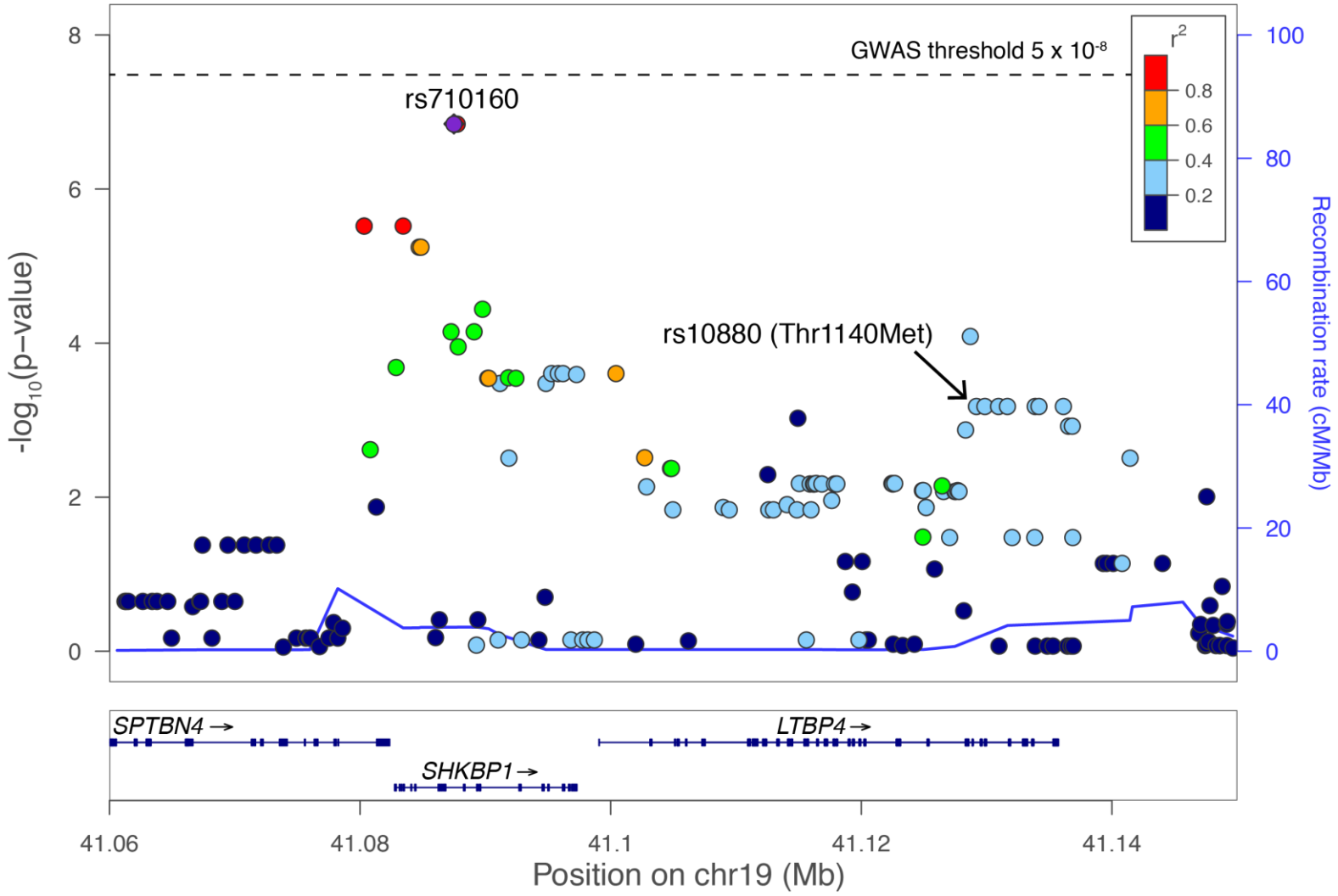
UDP cohort (n = 253), recessive genotype *P*-values of linear regression for age at loss of ambulation with **1,180,493 Illumina Omni2.5M SNPs** (MAF > 0.05)



CINRG Exome-Chip cohort (n=109), additive genotype *P*-values for age at loss of ambulation, **27,025 SNPs** (MAF > 0.05)

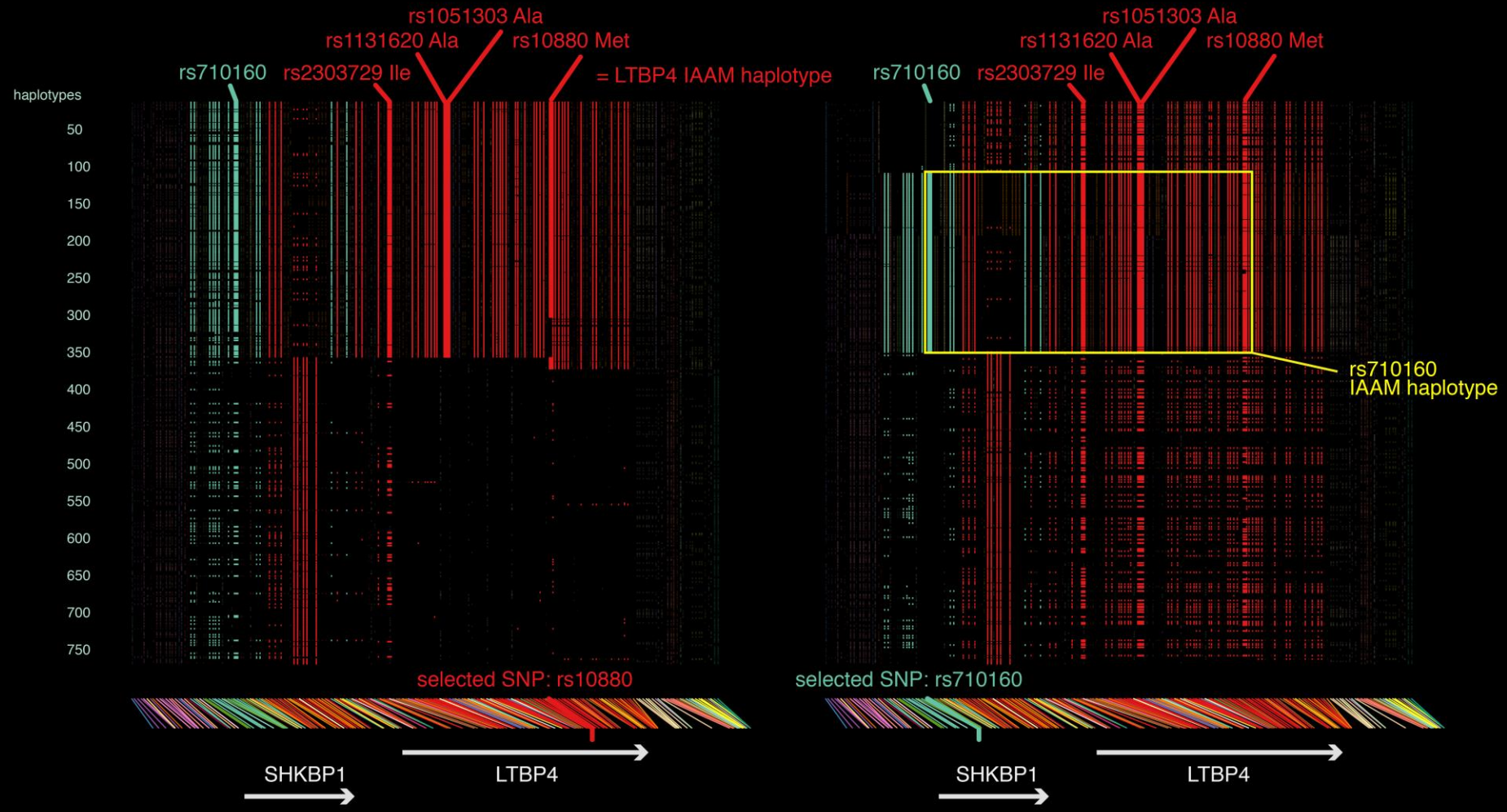


LTBP4 region single SNP associations

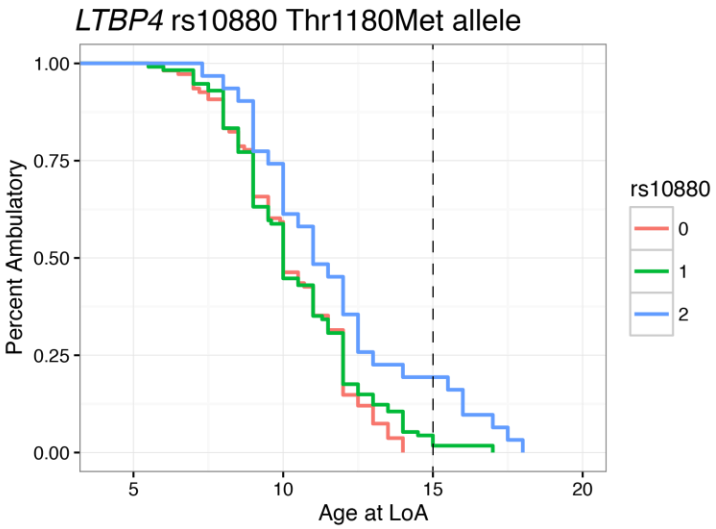


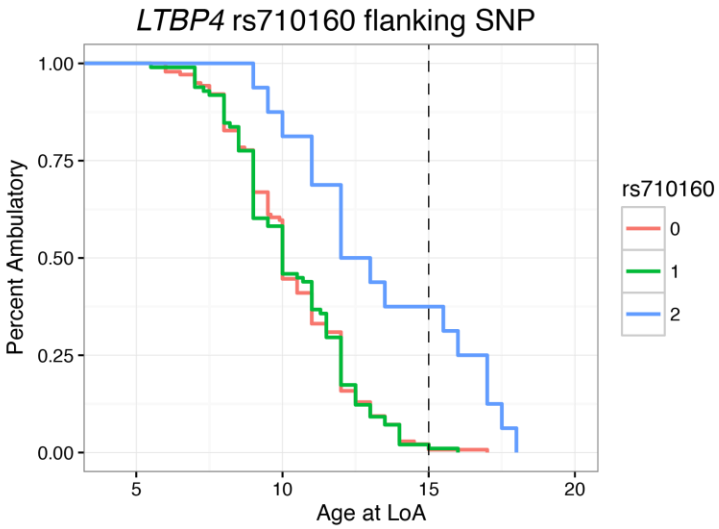
LTBP4 haplotype structure: 758 European reference chromosomes

SNPs colored by LD bin ($r^2 > 0.64$)



rs710160 is an expression QTL for *LTBP4* in fibroblasts

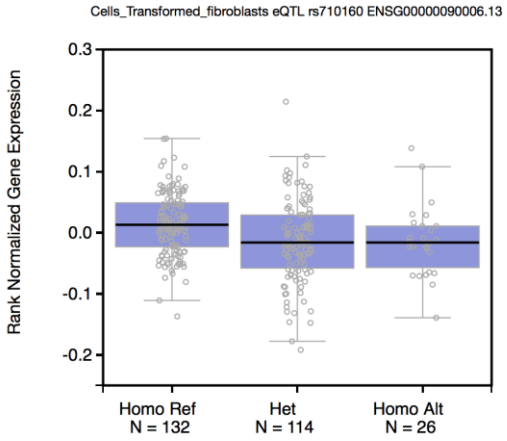




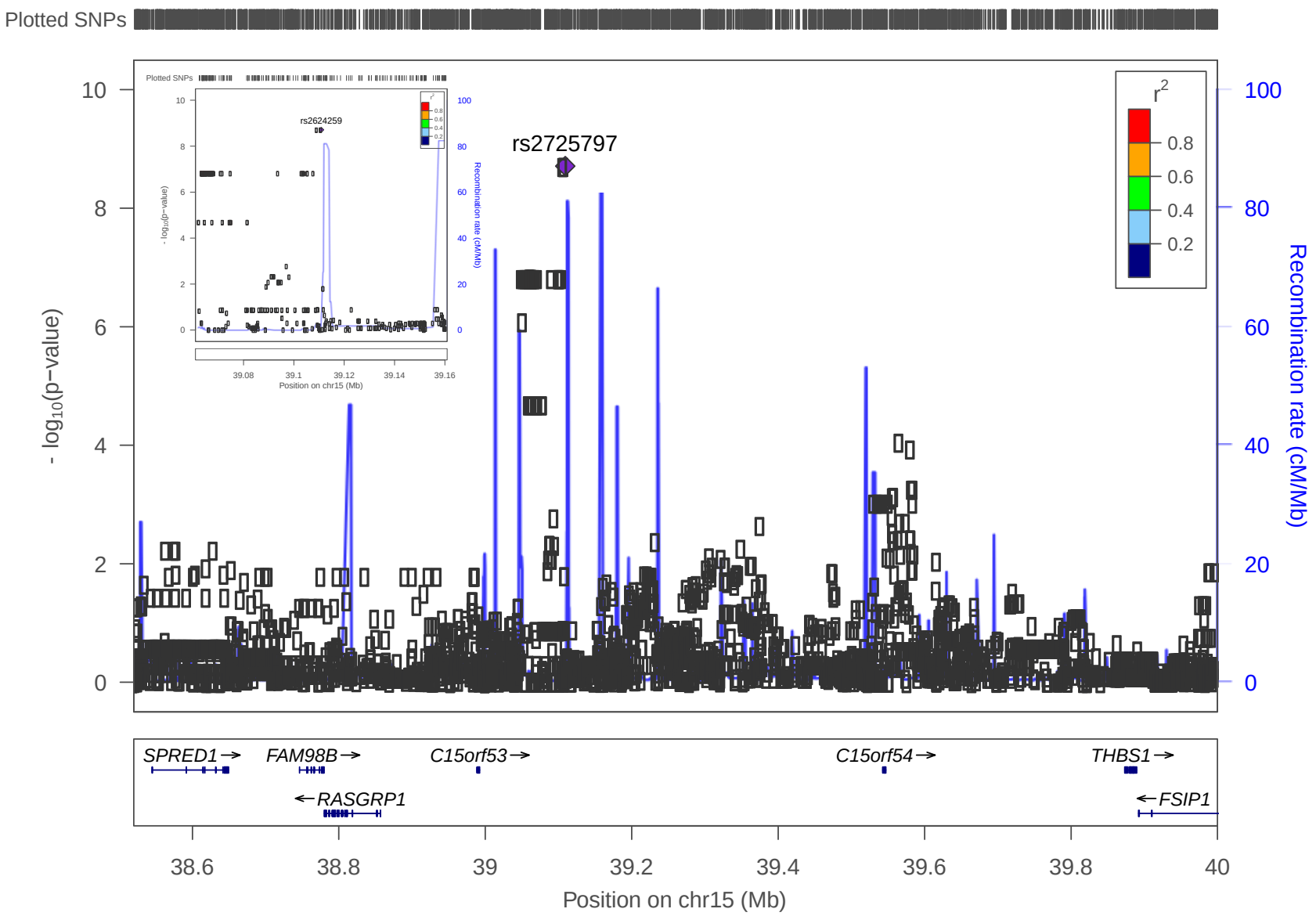
Genotype and RNA-Seq data (eQTL) in 43 tissues from
The Genotype-Tissue Expression (GTEx) Project

Gene Symbol	SNP	P-Value	Effect Size	Tissue
LTBP4	rs710160	0.00039	-0.13	Cells - Transformed fibroblasts
LTBP4	rs710160	0.04	0.33	Brain - Cerebellar Hemisphere

The Genotype-Tissue Expression (GTEx) pilot analysis: Multi-tissue gene regulation in humans. The GTEx Consortium. Science May 2015: 348, 6235, pp. 648-660



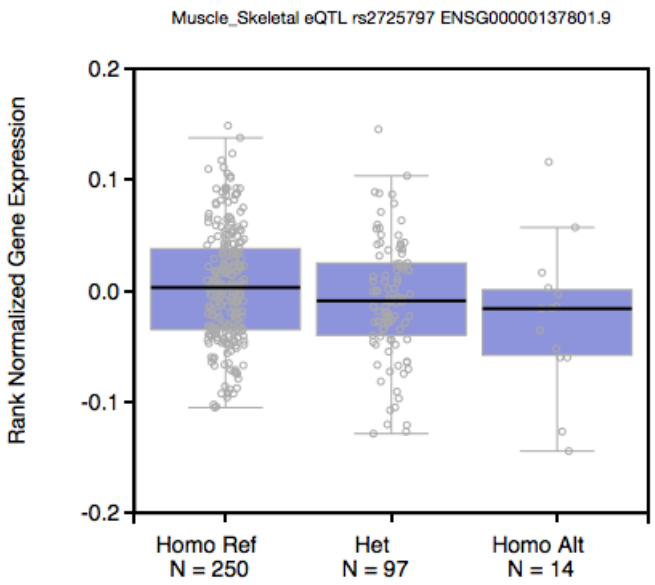
Top GWAS signal in the chr15 region

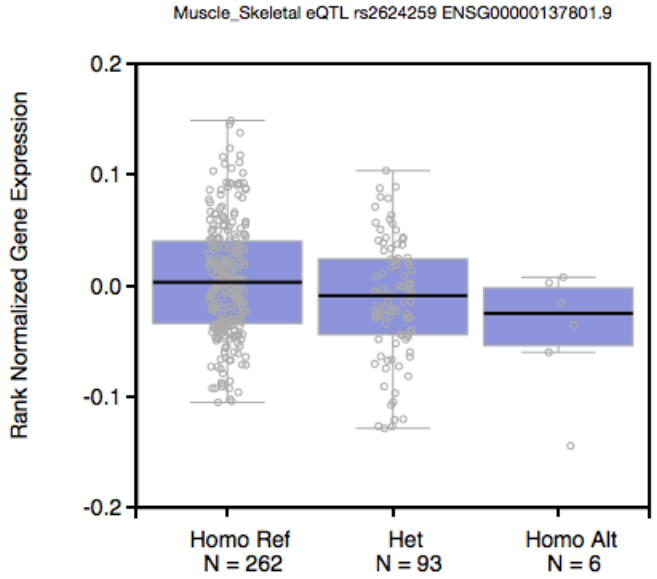


Chr15 regulatory SNPs are associated with decreased THBS1 mRNA in skeletal muscle

Genotype and tissue-specific gene expression from the GTEx Project

Tissue Rank (out of 41)	Gene Symbol	SNP	P-Value	Effect Size	Tissue (41 examined)
1	THBS1	rs2624259	0.00054	-0.24	Muscle - Skeletal
2	THBS1	rs2624259	0.036	-0.17	Esophagus - Mucosa
3	THBS1	rs2624259	0.074	-0.23	Spleen
4	THBS1	rs2624259	0.2	0.33	Brain - Anterior cingulate cortex (BA24)
5	THBS1	rs2624259	0.22	0.18	Artery - Coronary





Expression data from The Genotype-Tissue Expression (GTEx) Project

THBS1 ‘super-enhancer’ region

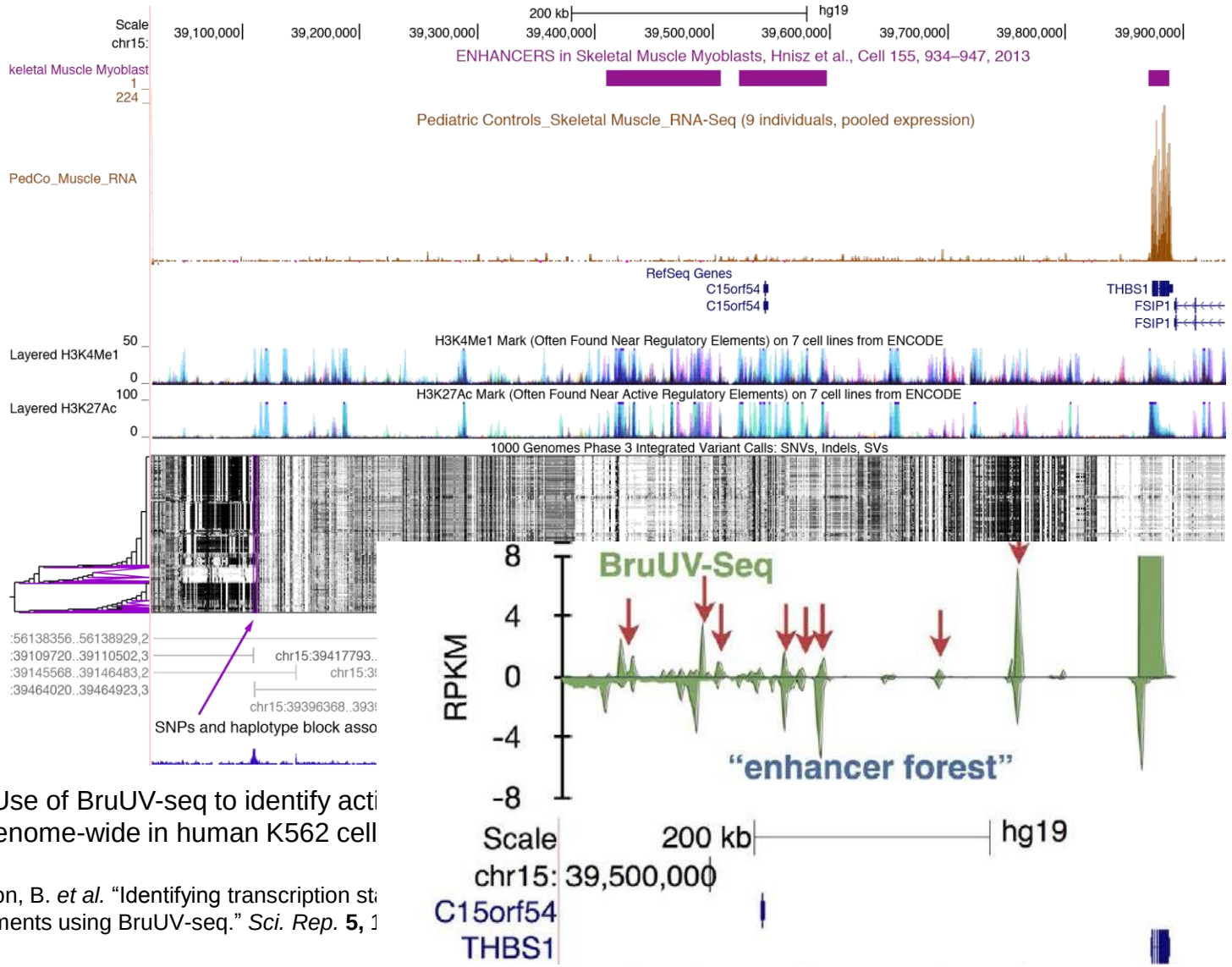
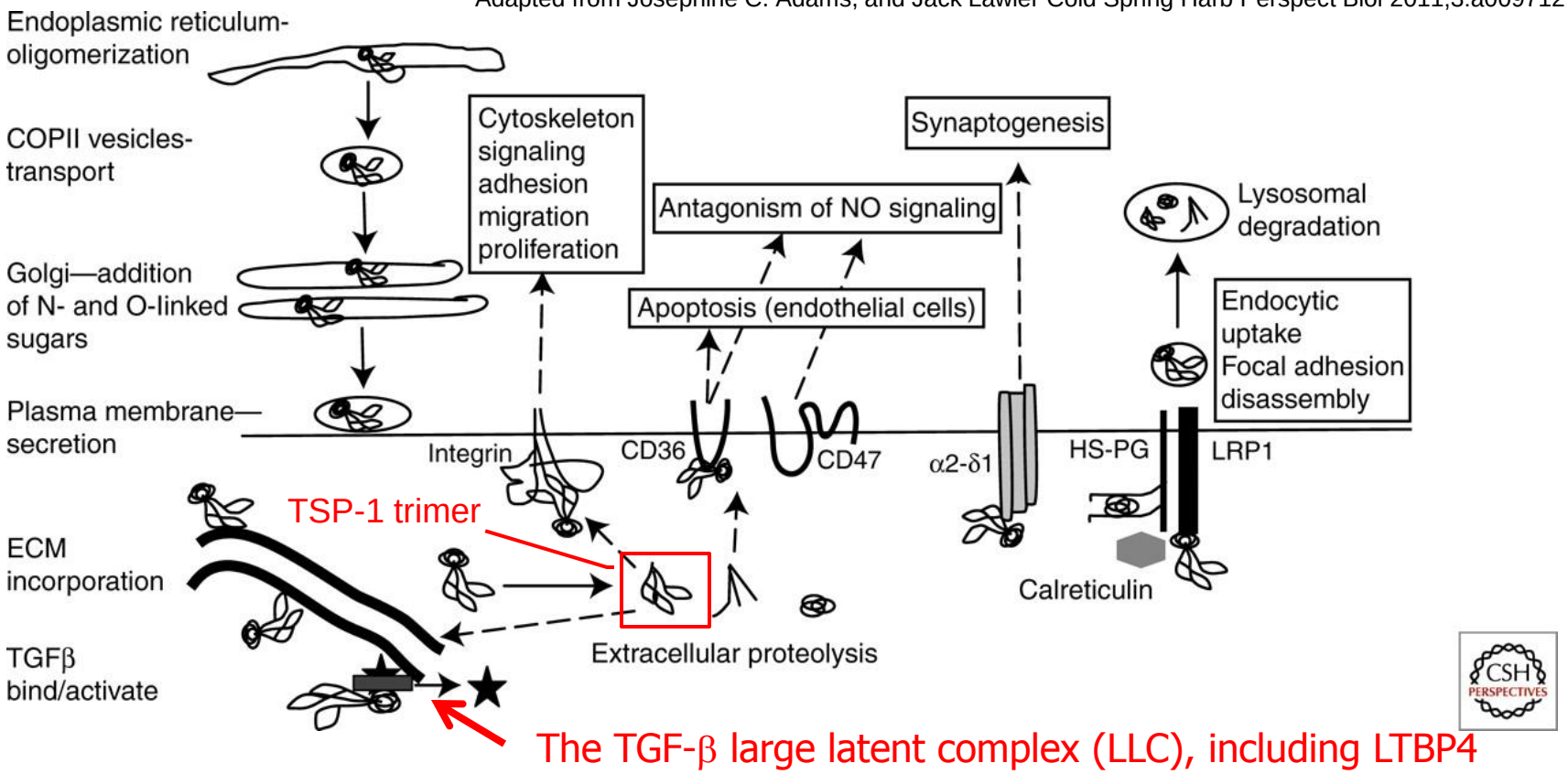


Figure 3b. Use of BruUV-seq to identify acti
elements genome-wide in human K562 cell

from Magnuson, B. *et al.* “Identifying transcription st
enhancer elements using BruUV-seq.” *Sci. Rep.* **5,** 1

Overview of cellular pathways and activities of mammalian TSP-1

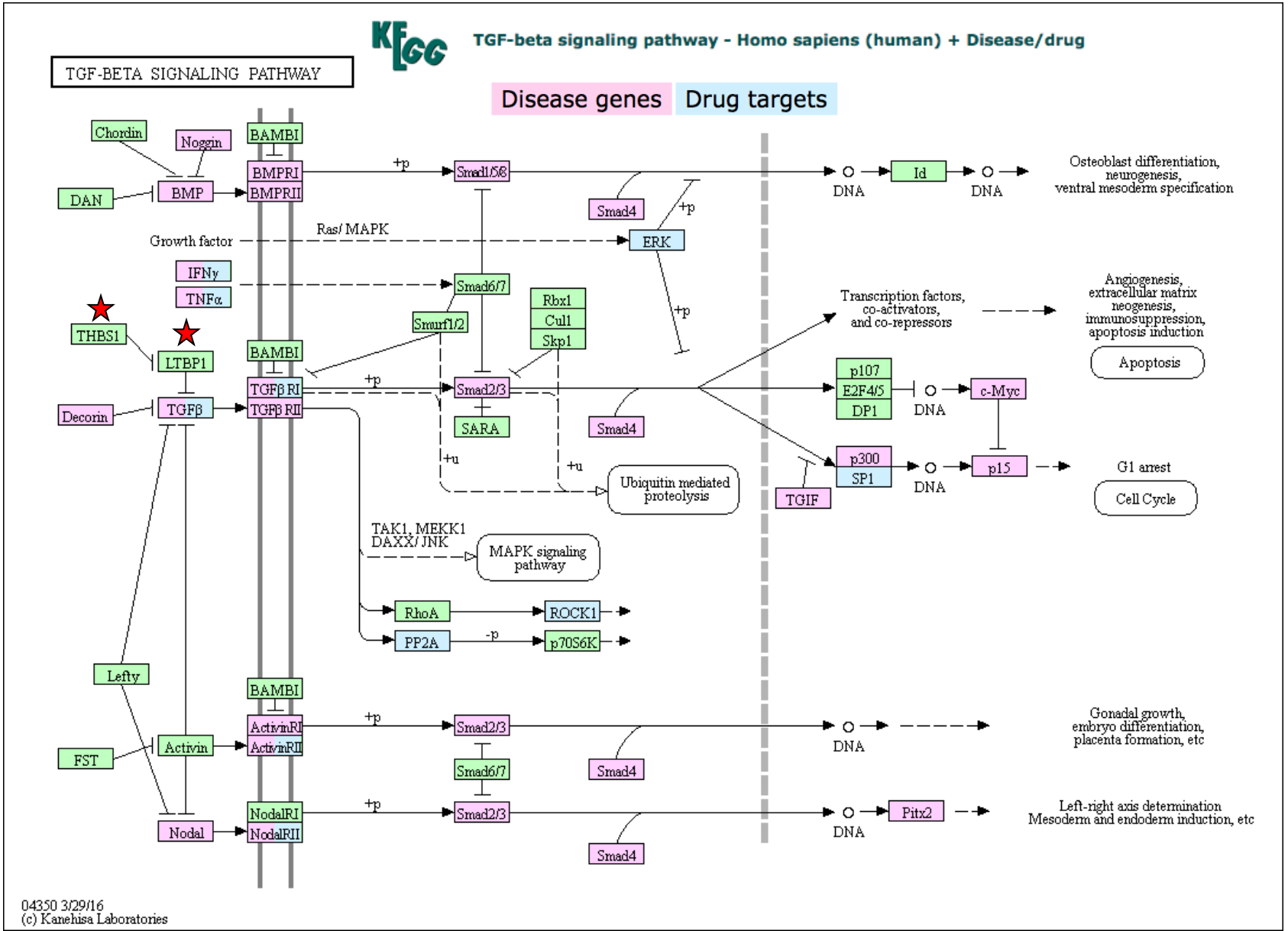
Adapted from Josephine C. Adams, and Jack Lawler Cold Spring Harb Perspect Biol 2011;3:a009712



Schultz-Cherry, S. & Murphy-Ullrich, J. E. Thrombospondin causes activation of latent transforming growth factor-beta secreted by endothelial cells by a novel mechanism. *J Cell Biol* **122,** 923–932 (1993).

Zhou, Y. *et al.* Latent transforming growth factor-beta-binding protein-4 regulates transforming growth factor-beta1 bioavailability for activation by fibrogenic lung fibroblasts in response to bleomycin. *Am. J. Pathol.* **174,** 21–33 (2009).

Summary: modifier genes and TGF-β signaling



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Summary

- Genetic variants that modulate TGF-β signaling delay disease progression in DMD
- *LTBP4* and *CD40* variants have replicated effects across multiple cohorts
- Initial GWAS results confirm the strength of the *LTBP4* effect and suggest that other large effect variants exist.

UPIN UTAH PROGRAM FOR INHERITED NEUROMUSCULAR DISORDERS

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Craig McDonald

CINRG-DNHS

Luca Bello, Eric Hoffman

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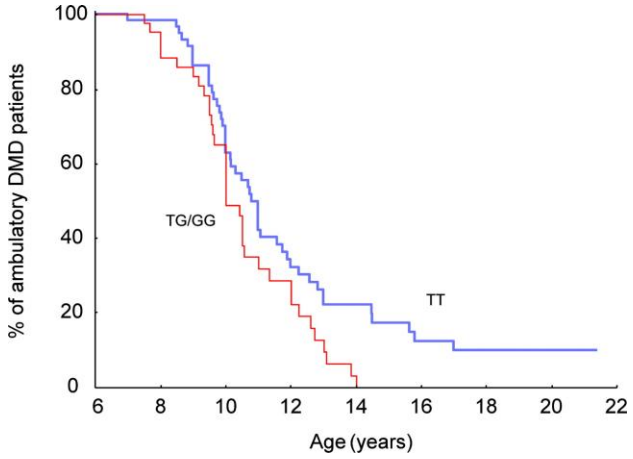
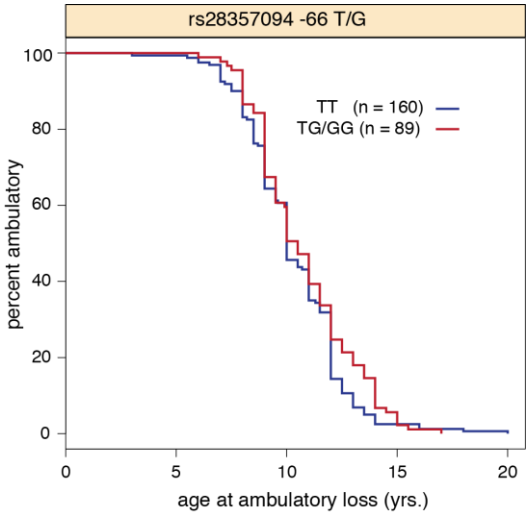
University of Padova, Italy

Osteopontin (*SPP1)* Replication Studies

Figure 3 (Pegoraro *et al.*) *SPP1* genotype is associated with greater severity of progression in Duchenne muscular dystrophy (DMD). The proportion of patients with DMD in the Padova cohort remaining ambulatory at the specific age noted is shown (n = 106).



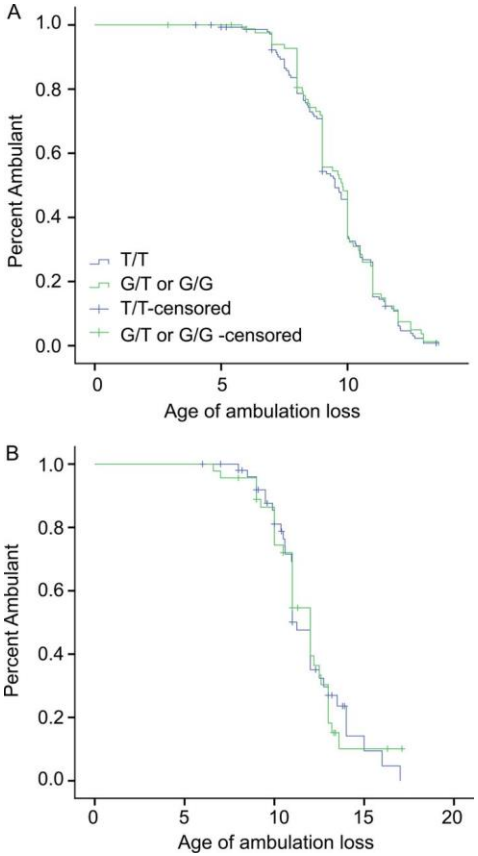
Supplementary Table S9. *SPP1* rs28357094 -66 T/G promoter SNP is not associated with age at ambulatory loss with steroid treatment and *DMD* mutation class as covariates in 239 patients. from Flanigan et al., Ann Neurol. 73(4):481-488 (2013).



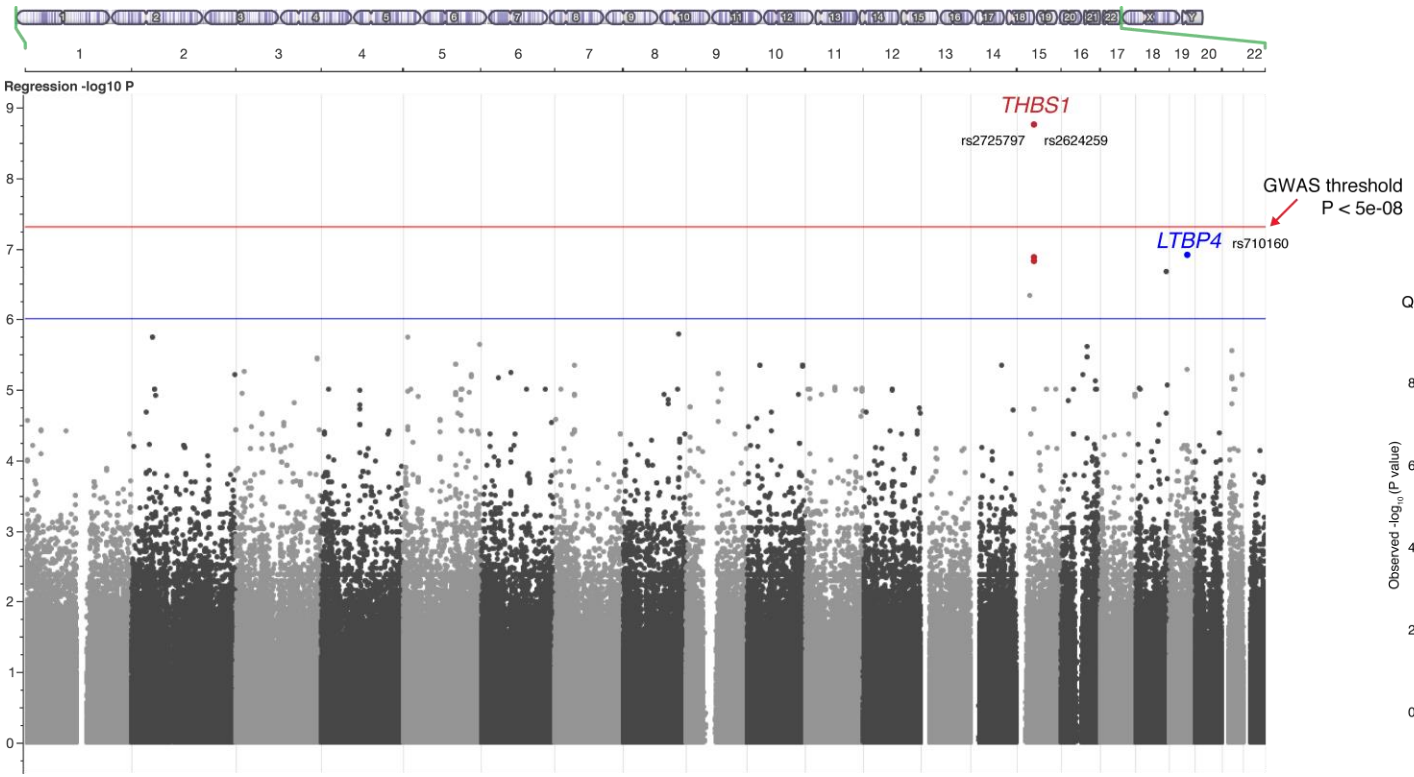
Pegoraro, E., E. P. Hoffman, L. Piva, B. F. Gavassini, S. Cagnin, M. Ermani, L. Bello, et al. 2011. "SPP1 Genotype Is a Determinant of Disease Severity in Duchenne Muscular Dystrophy." *Neurology* 76 (3): 219–26.

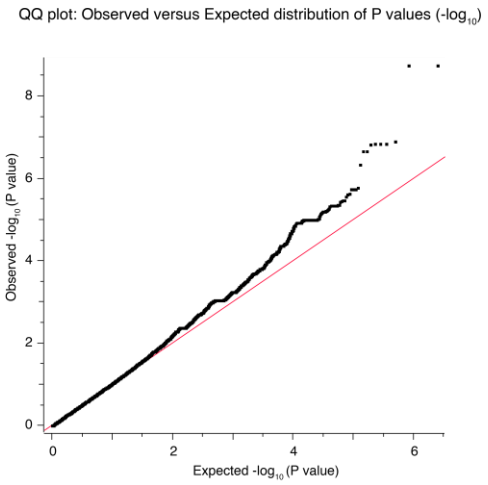
rs28357094 is in the promoter of *SPP1* (osteopontin) and the G allele (dominant model) was associated with more rapid progression (Padova cohort log rank p = 0.003), and 12%–19% less grip strength (CINRG cohort p = 0.0003).

Figure 1: Survival plots showing the effect of single nucleotide polymorphism (SNP) rs28357094 (SPP1) for 336 patients with Duchenne muscular dystrophy (DMD). From JC van den Bergen et al. "Validation of genetic modifiers for Duchenne muscular dystrophy: a multicentre study assessing SPP1 and LTBP4 variants" J Neurol Neurosurg Psychiatry 2015;86:1060-1065



Genome-Wide Association Study (GWAS) of Age at Ambulatory Loss: 252 patients



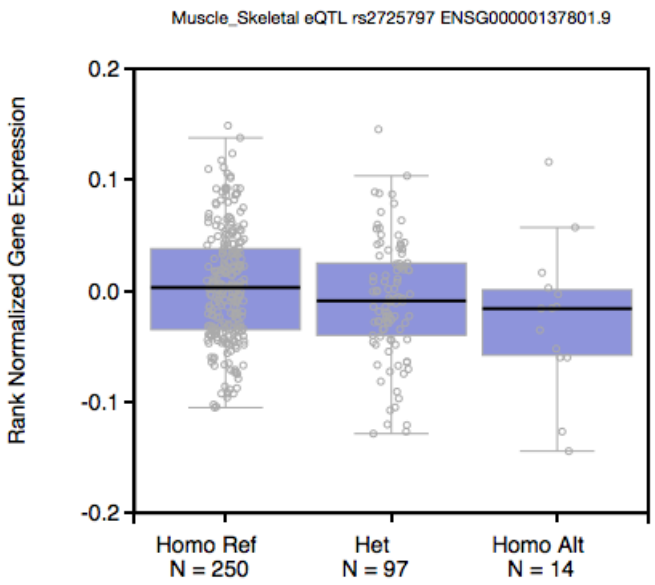


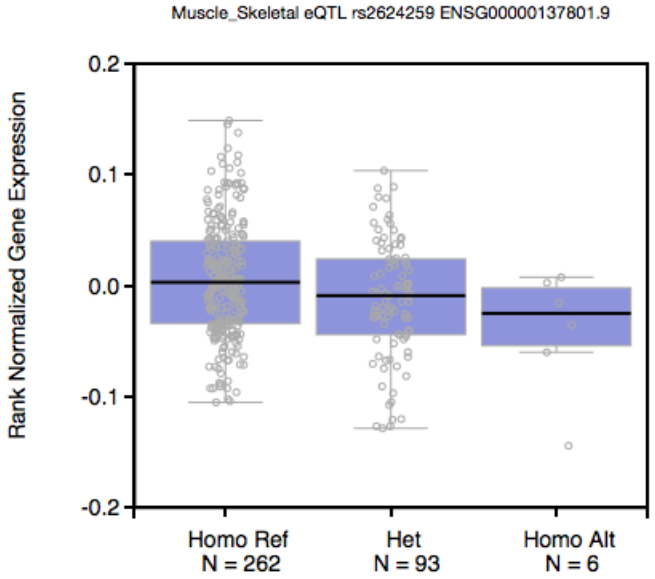
Regression P values: 1,289,642 SNPs, maf > 0.05, recessive model
Top SNPs = rs2725797 and rs2624259, P = 1.8e-09

Genotyping Platform = Illumina Omni2.5-Exome array

rs2725797 & rs2624259 genotype effects : minor allele associated with decreased THBS1 mRNA expression in skeletal muscle

Gene Symbol	SNP	P-Value	Effect Size	Tissue
THBS1	rs2624259	0.00054	-0.24	Muscle - Skeletal
THBS1	rs2624259	0.036	-0.17	Esophagus - Mucosa
THBS1	rs2624259	0.074	-0.23	Spleen
THBS1	rs2624259	0.2	0.33	Brain - Anterior cingulate cortex (BA24)
THBS1	rs2624259	0.22	0.18	Artery - Coronary





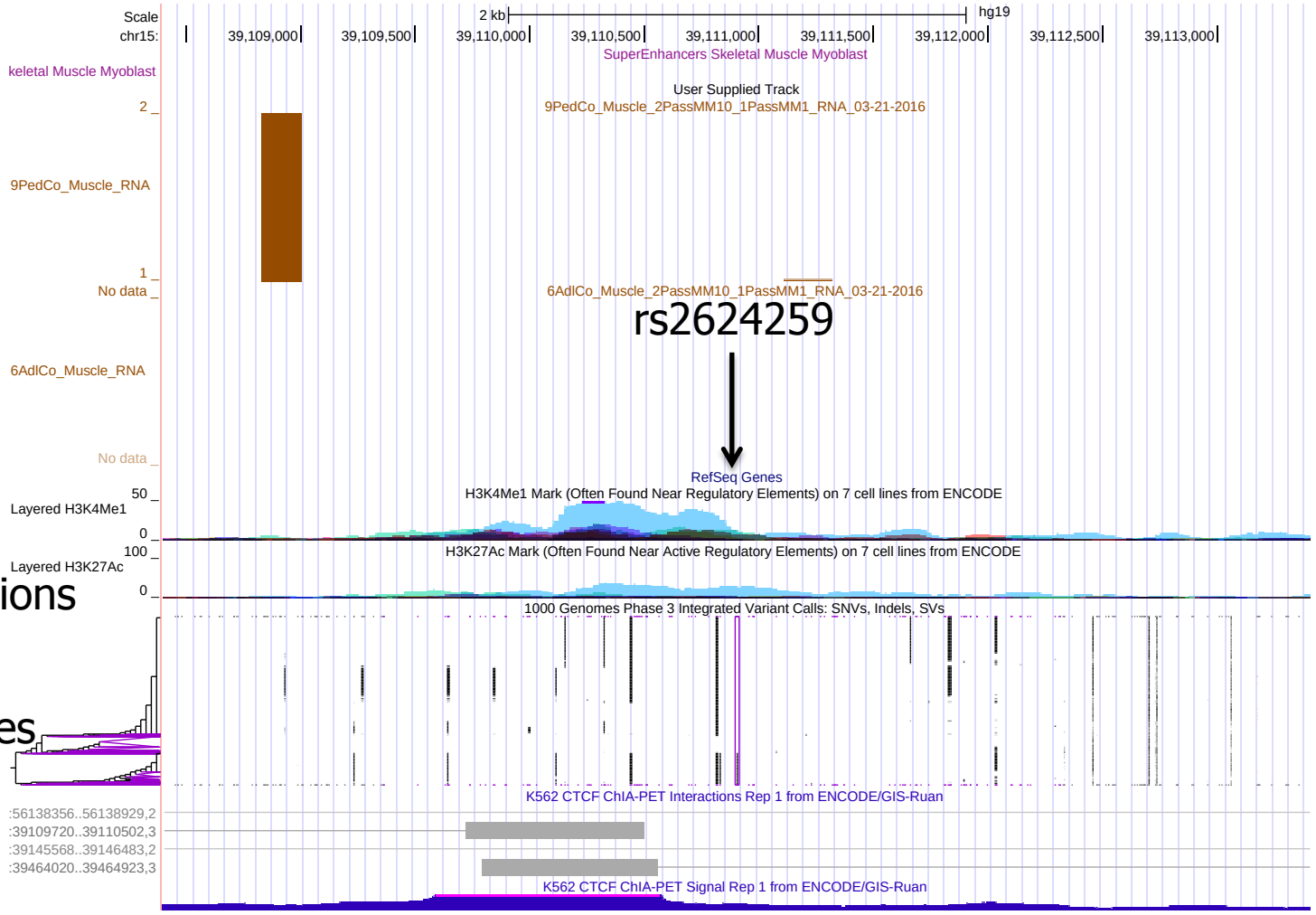
Expression data from The Genotype-Tissue Expression (GTEx) Project

Chromatin Features

RNA-Seq

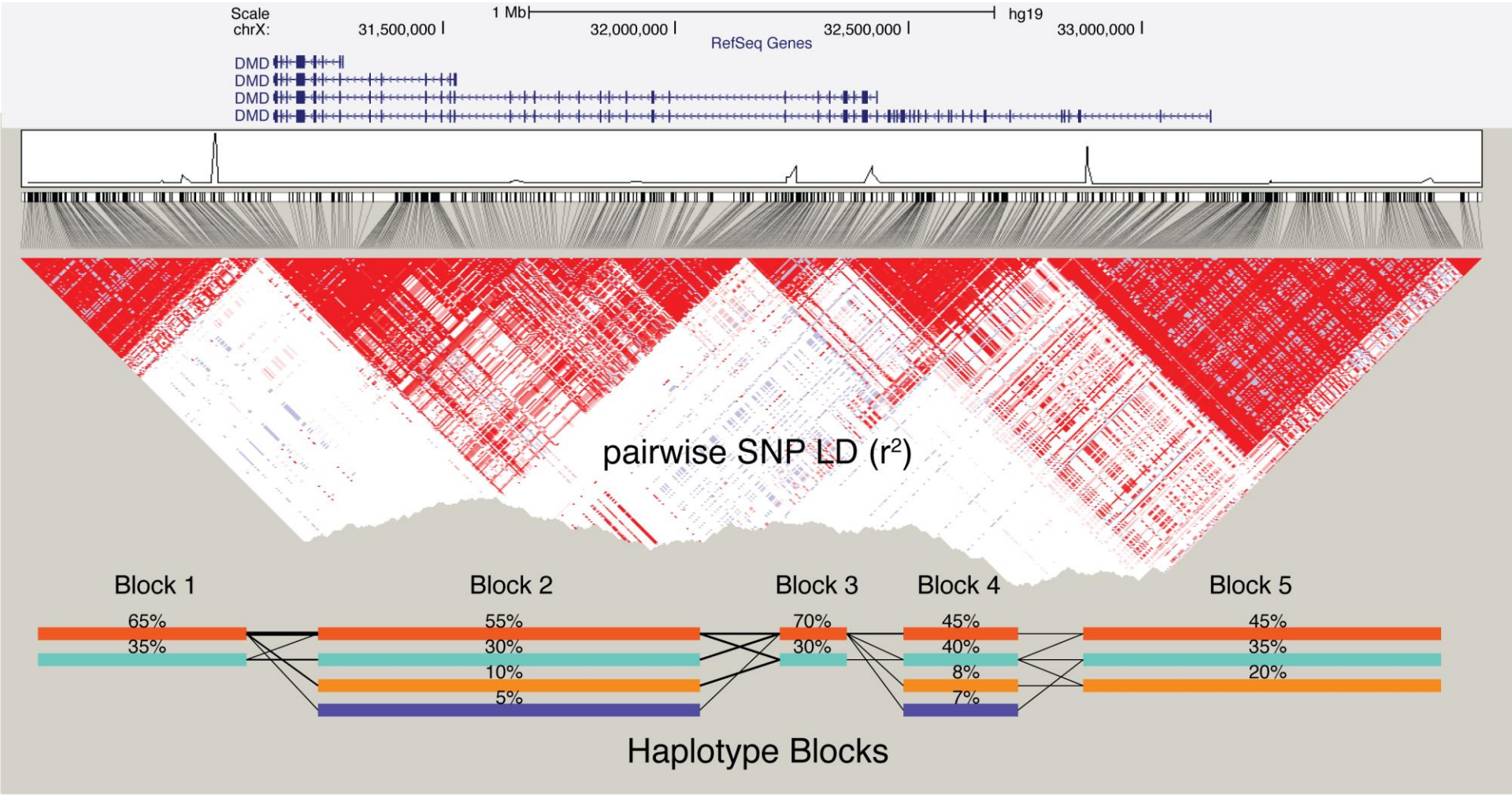
Histone modifications

Haplotypes

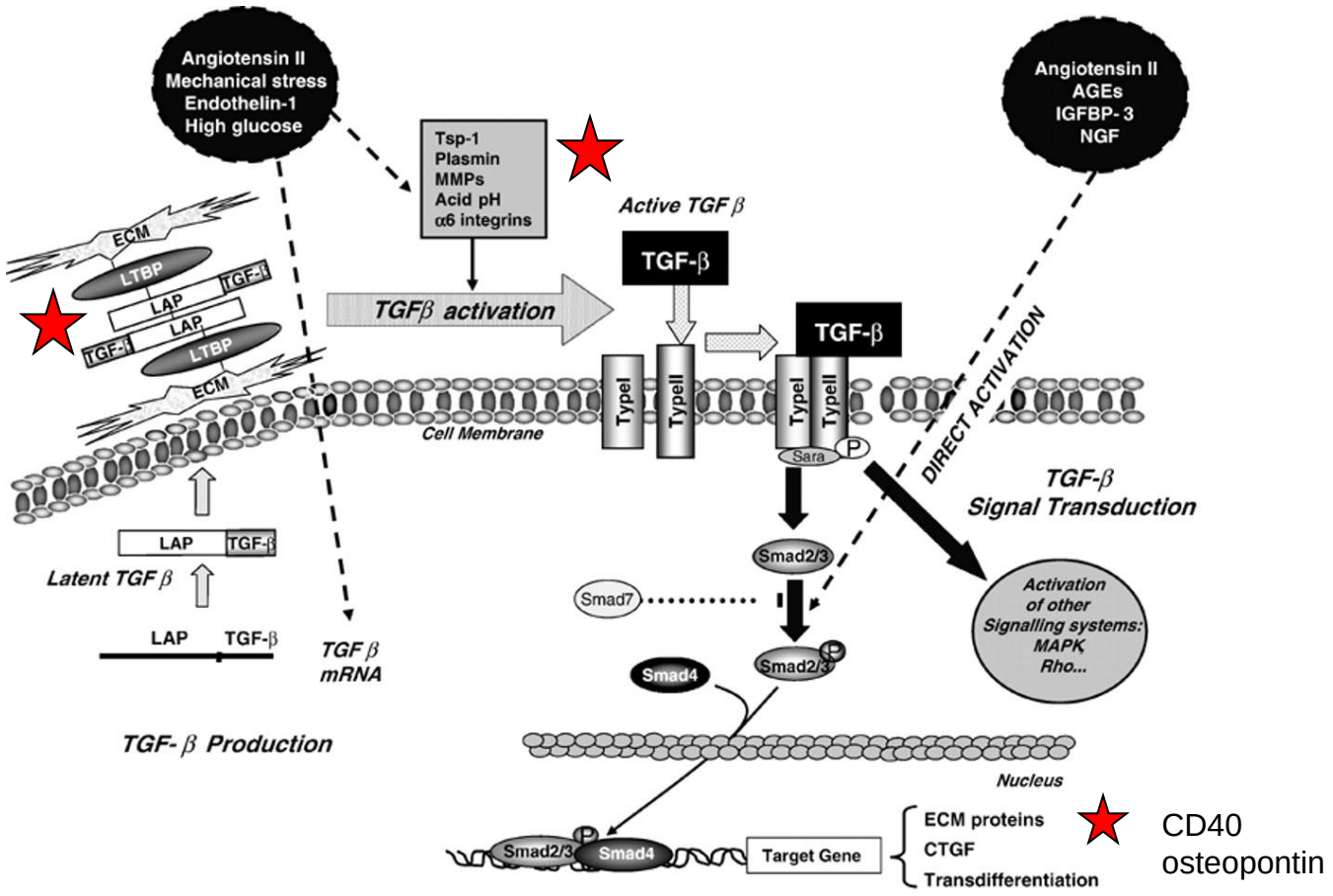


chromatin interaction analysis by paired-end tag sequencing (ChIA-PET)

Haplotypes and LD structure



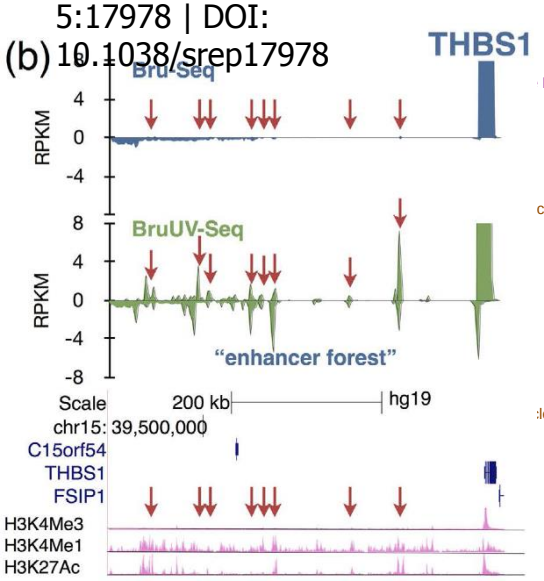
DMD modifier genes and TGF-β signaling

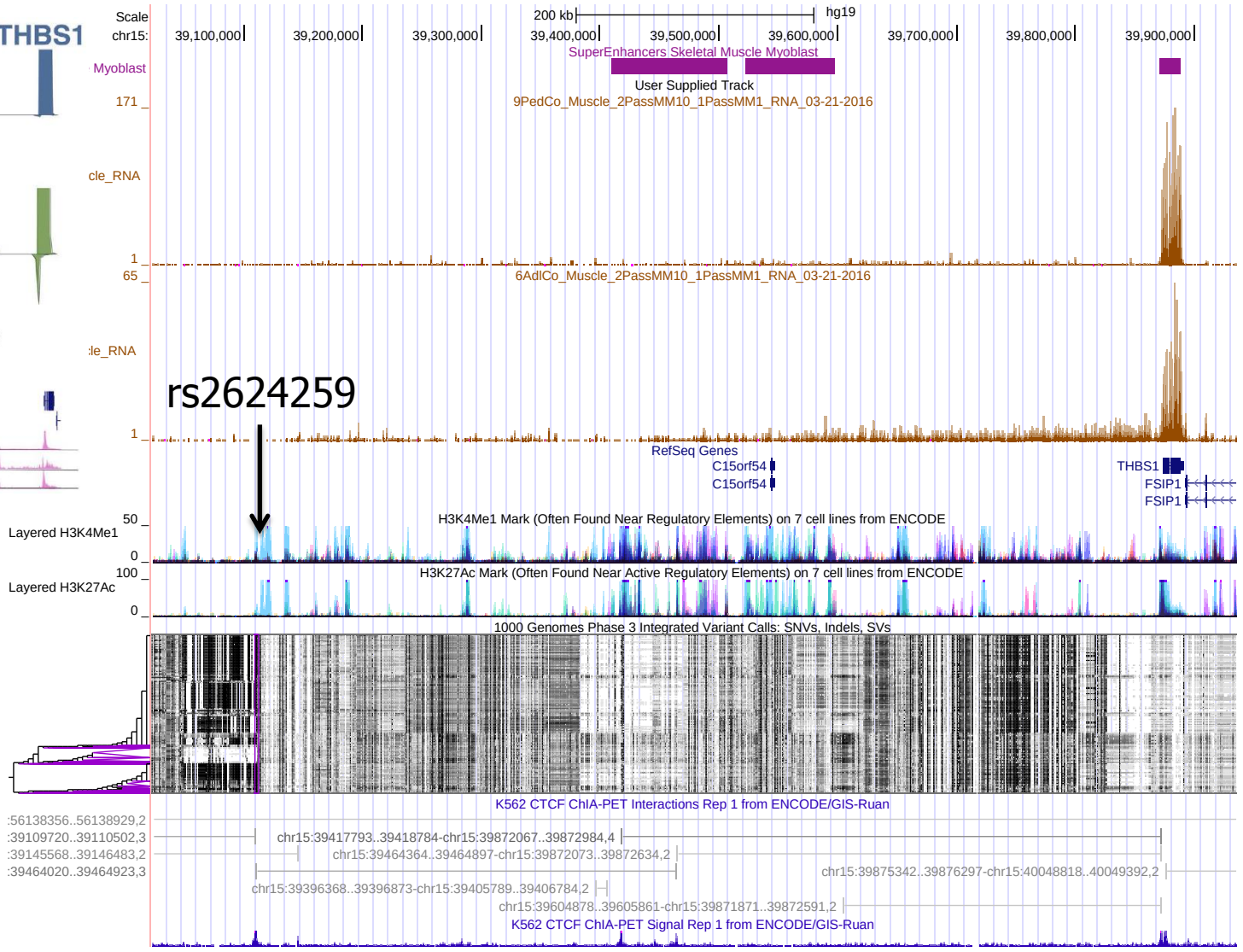


adapted from Marta Ruiz-Ortega et al. Cardiovasc Res 2007;74:196-206

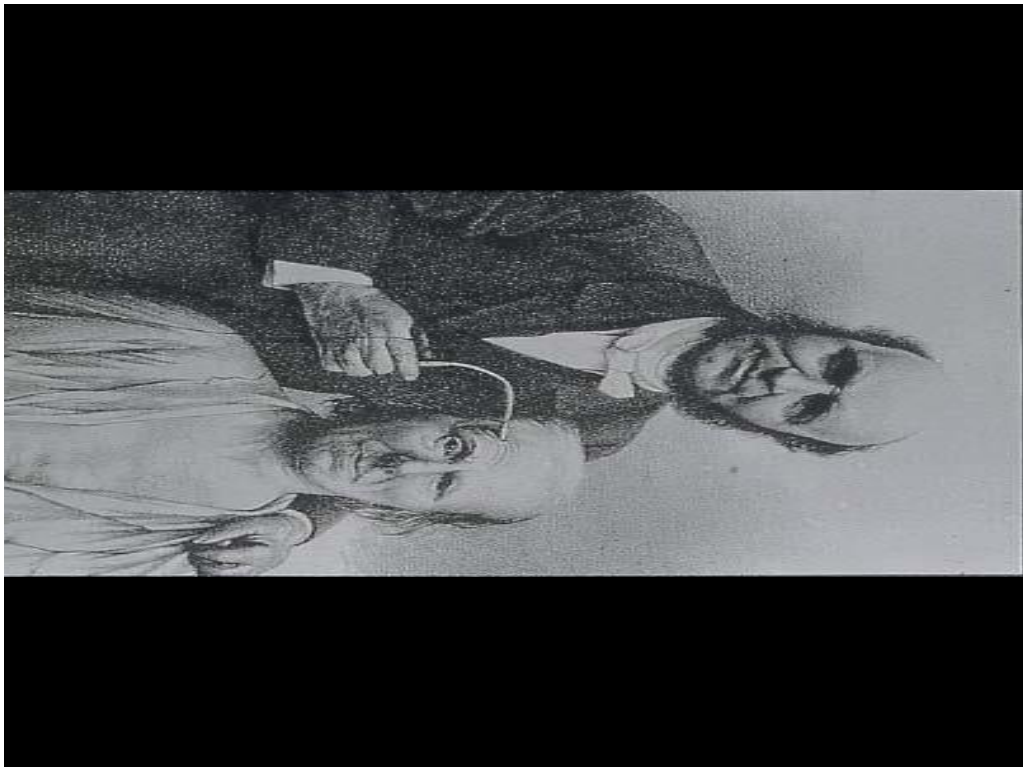
Identifying transcription start sites and active enhancer elements using
BruUV-seq. Scientific Reports | 5:17978 | DOI: 10.1038/srep17978

THBS1 'Super Enhancer'





Guillaume Benjamin Duchenne, pioneer in neurology, 1806-1875



In his publications of 1861 and 1868, the French physician Duchenne described in considerable detail the muscle disease that would bear his name. However, an English physician, Edward Meryon described the disorder some 10 years early and published the details in English.

RESEARCH ARTICLE

Human Mutation

OFFICIAL JOURNAL
HGVS
HUMAN GENOME VARIATION SOCIETY
www.hgvs.org

Mutational Spectrum of DMD Mutations in Dystrophinopathy Patients: Application of Modern Diagnostic Techniques to a Large Cohort

Kevin M. Flanigan,[1–4]* Diane M. Dunn,[1] Andrew von Niederhausern,[1] Payam Soltanzadeh,[1] Eduard Gappmaier,[1] Michael T. Howard,[1] Jacinda B. Sampson,[2] Jerry R. Mendell,[5] Cheryl Wall,[5] Wendy M. King,[5] Alan Pestronk,[6,7] Julaine M. Florence,[6] Anne M. Connolly,[6] Katherine D. Mathews,[8] Carrie M. Stephan,[8] Karla S. Laubenthal,[1,8] Brenda L. Wong,[9,10] Paula J. Morehart,[10] Amy Meyer,[10] Richard S. Finkel,[11,12] Carsten G. Bonnemann,[11,12] Livija Medne,[11] John W. Day,[13] Joline C. Dalton,[13] Marcia K. Margolis,[13] Veronica J. Hinton,[14] the United Dystrophinopathy Project Consortium,† and Robert B. Weiss[1]*

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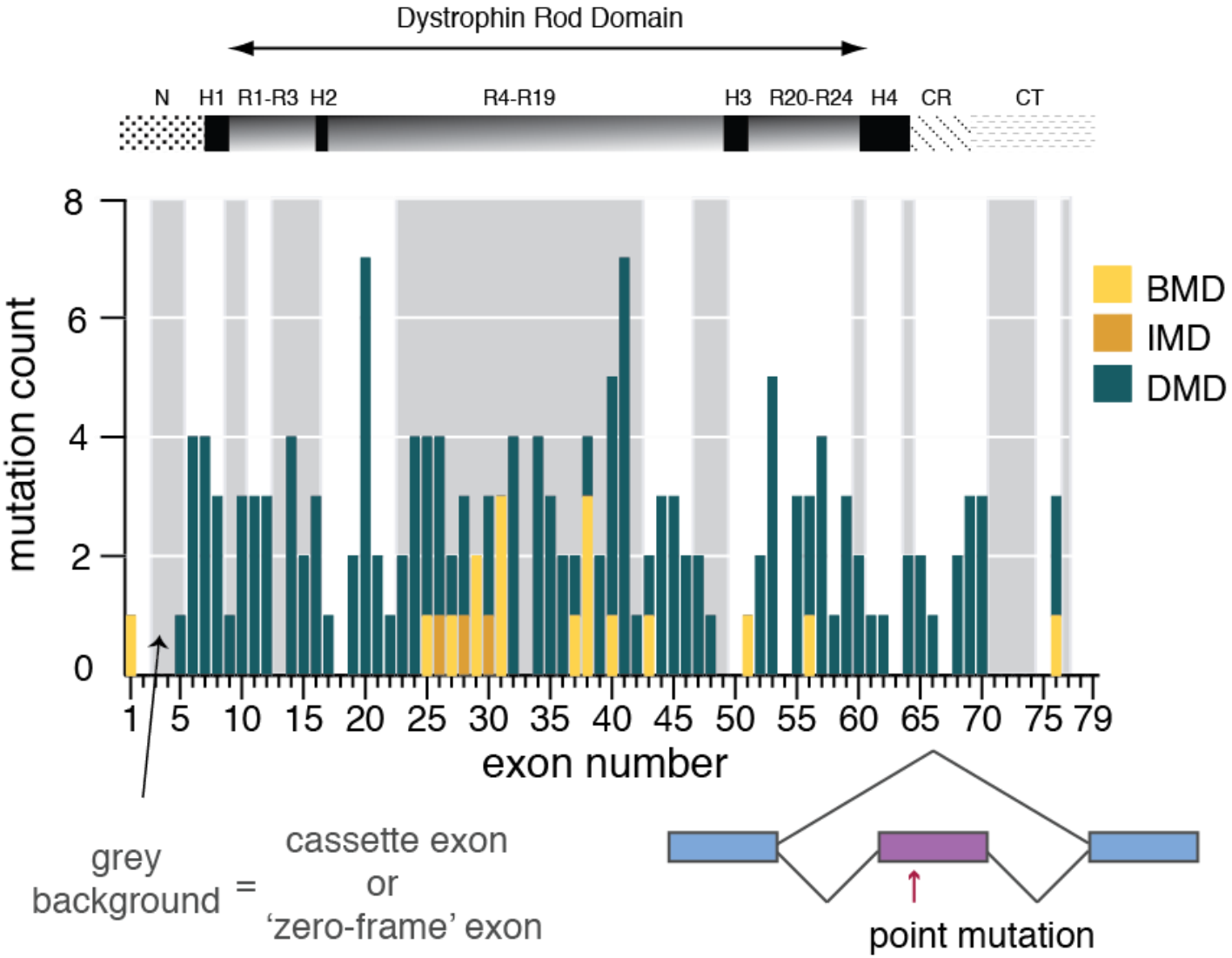
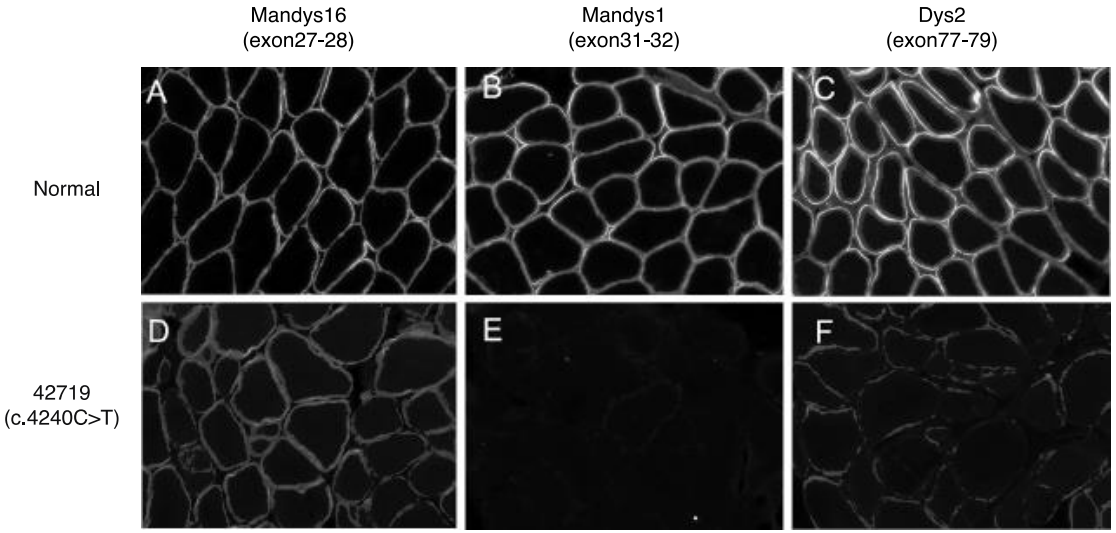
1,111 mutation-positive patients

Nonsense mutations: location versus phenotype

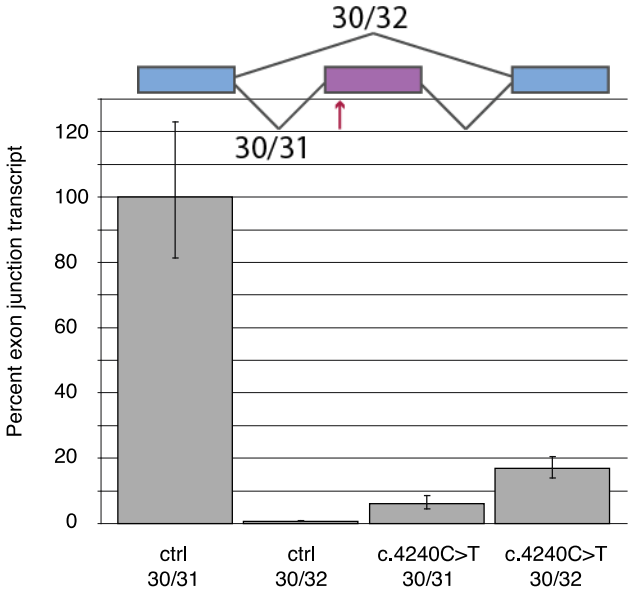


Becker Patient: Exon 31 c.4240C>T, p.Gln1414X

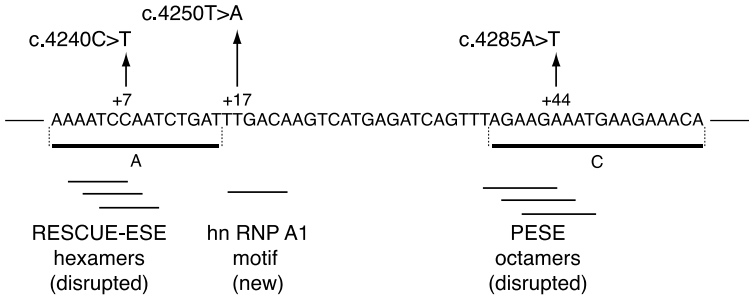
A

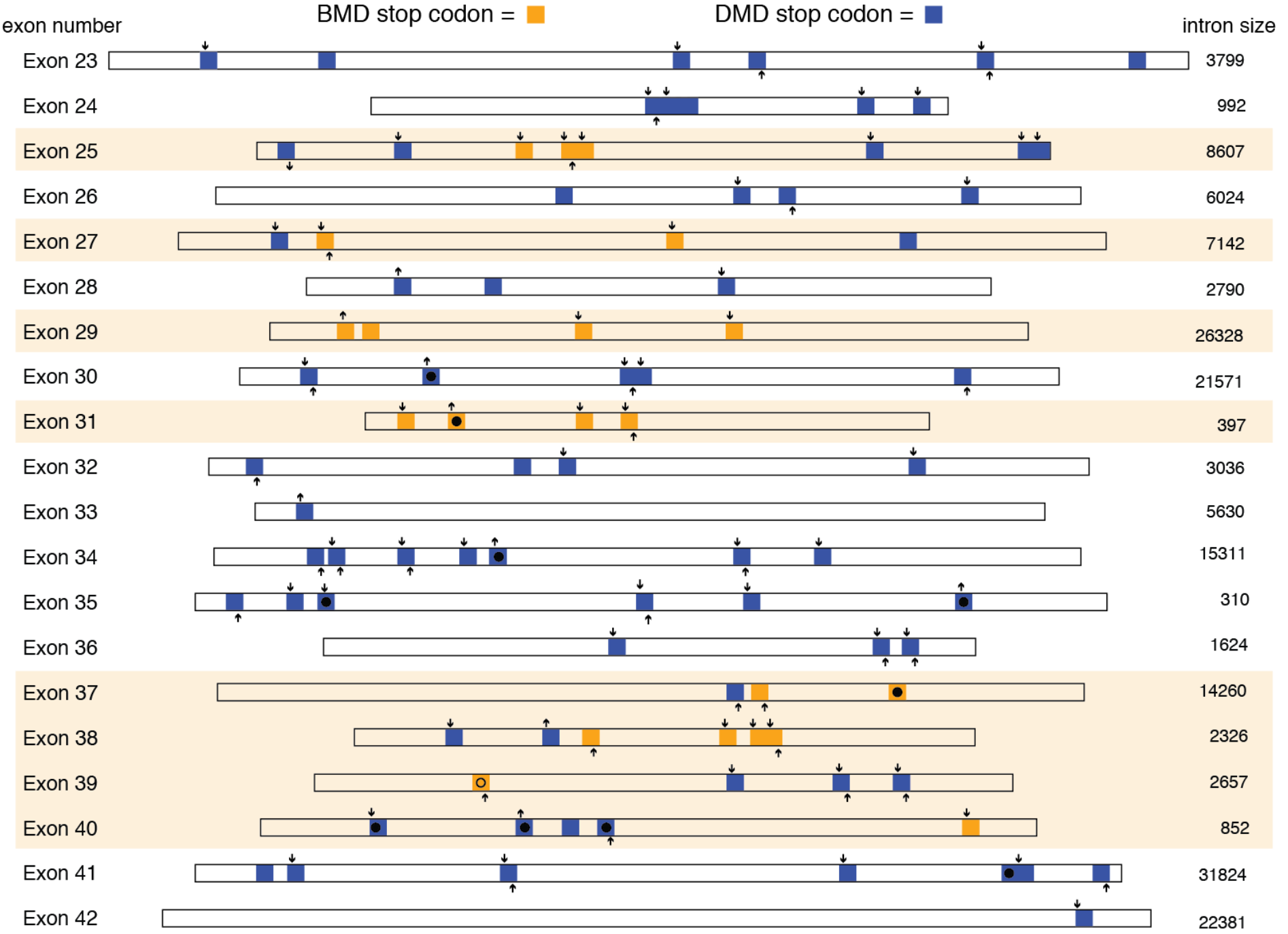


B



C

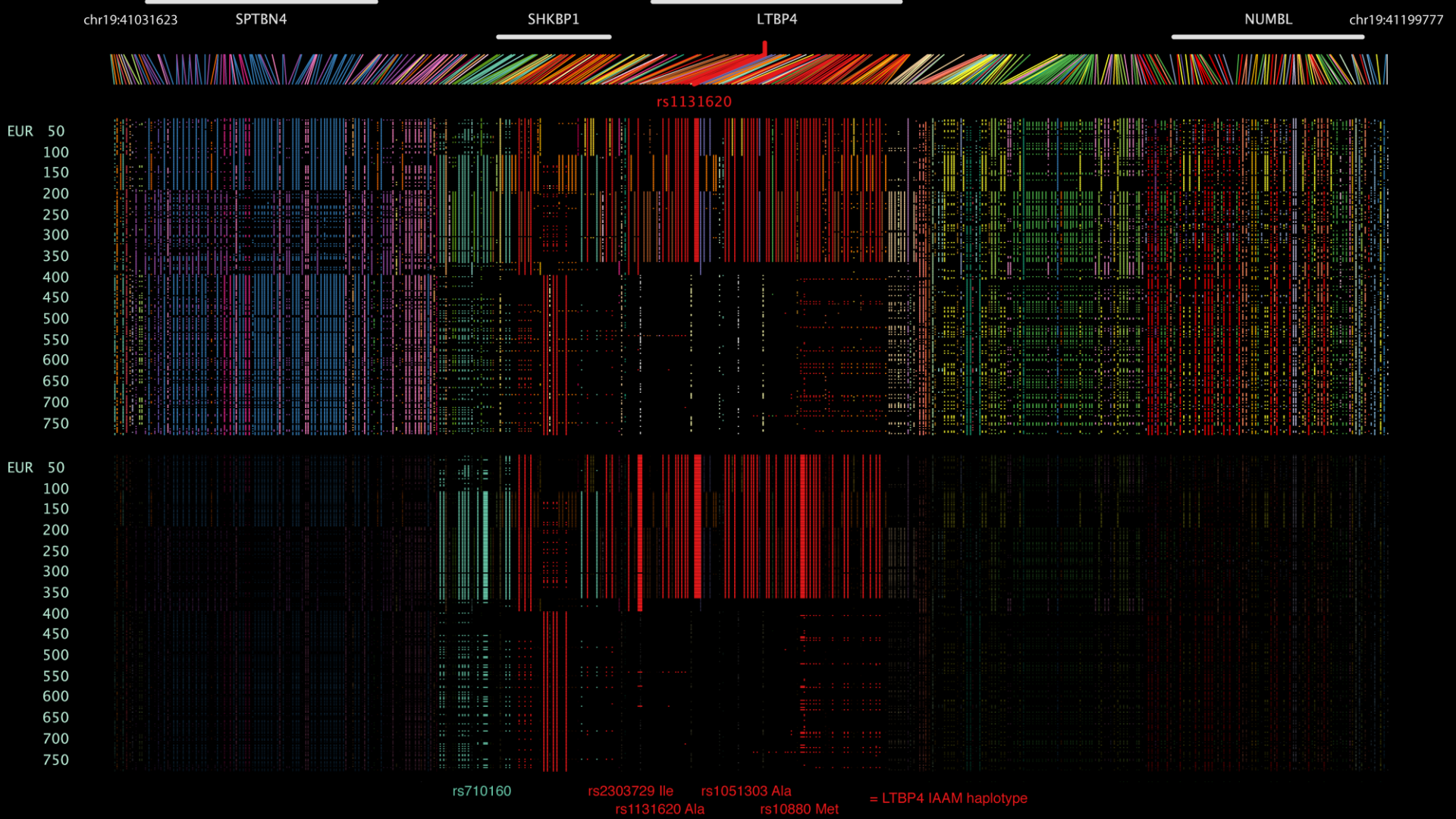



BMD stop codon =
DMD stop codon =
exon number
intron size
Exon 23
3799
Exon 24
992
Exon 25
8607
Exon 26
6024
Exon 27
7142
Exon 28
2790
Exon 29
26328
Exon 30
21571
Exon 31
397
Exon 32
3036
Exon 33
5630
Exon 34
15311
Exon 35
310
Exon 36
1624
Exon 37
14260
Exon 38
2326
Exon 39
2657
Exon 40
852
Exon 41
31824
Exon 42
22381

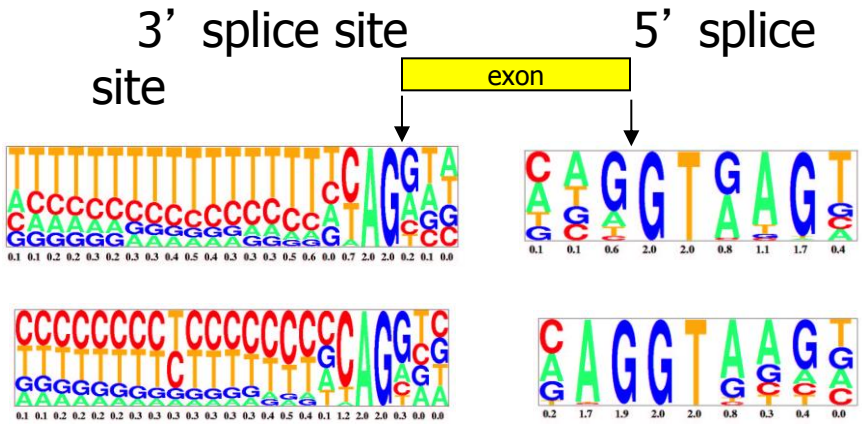
Genetic Association Studies in Humans

- Many genes
  - ~25,000 genes, many can be candidates
- Many SNPs
  - ~6,000,000 SNPs (MAF > 1%), ability to predict functional SNPs is limited
- Methods to select SNPs:
  - Only functional SNPs in a candidate gene
  - Systematic screen of SNPs in a candidate gene
  - Systematic screen of SNPs in an entire pathway
  - Genome-wide screen

LTBP4 haplotype structure: 758 European reference chromosomes

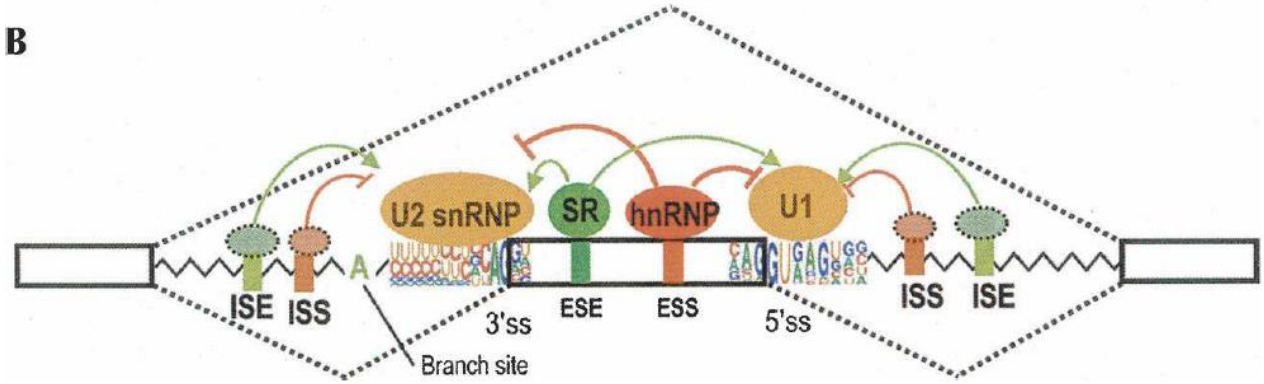


3' (acceptor) and 5' (donor) splice sites



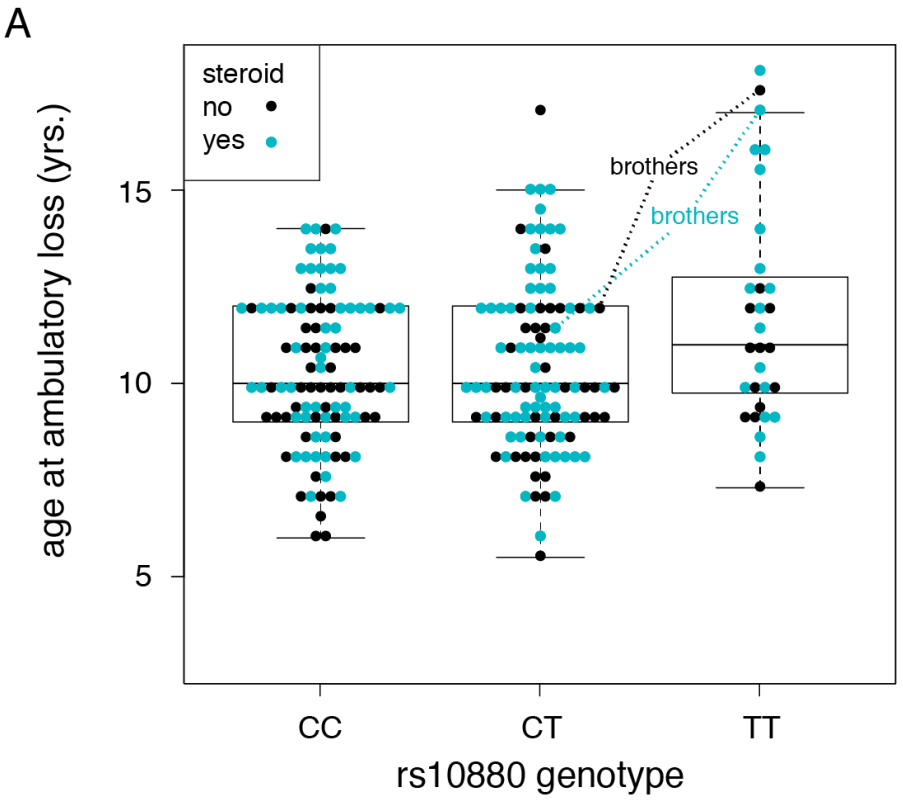
Scoring of splice site sequence motifs:

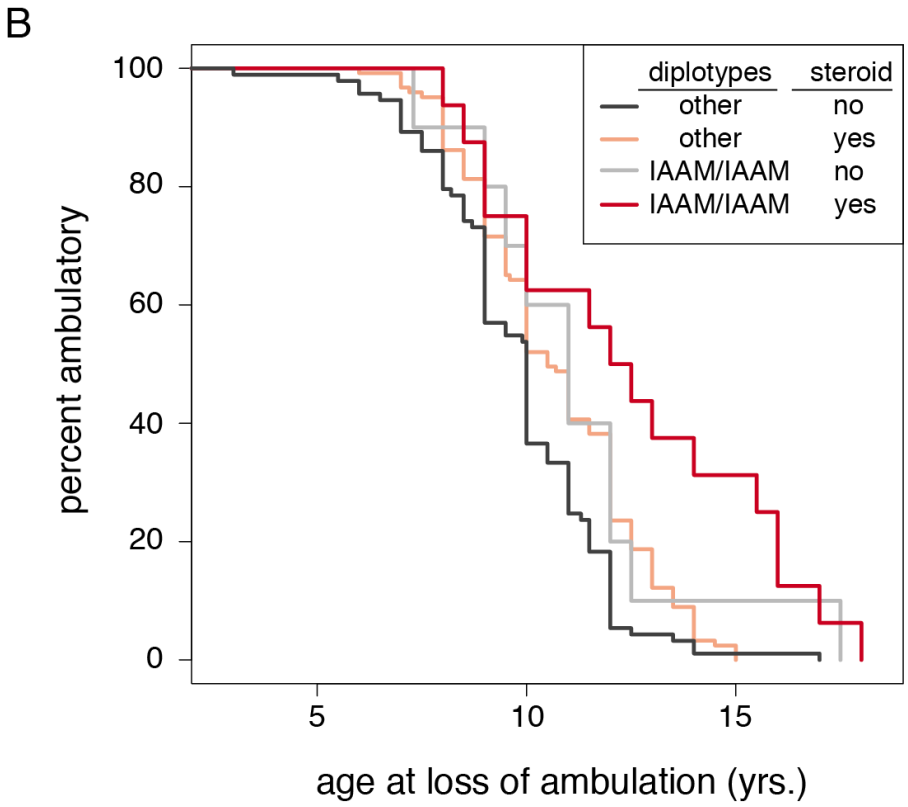
1. Weight Matrix Model (WMM)
2. First-order Markov Model (MM)
3. Maximum Dependence Decomposition Model (MDD)
4. Maximum Entropy Model (MaxENT)



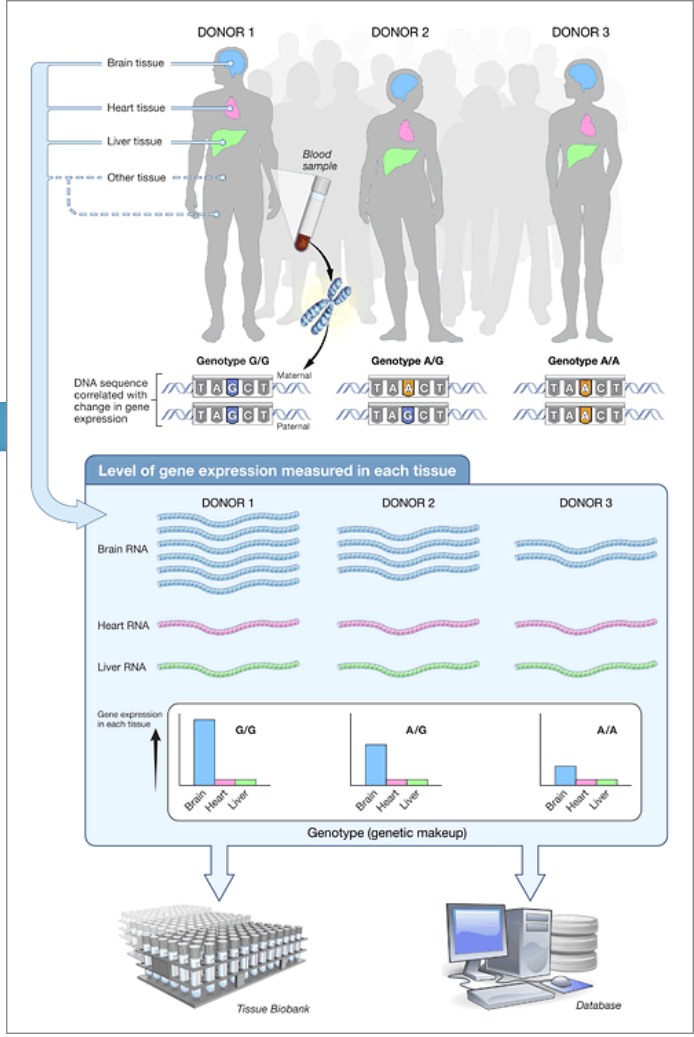
From Z. Wang & C. Burge, Splicing regulation: From a parts list of regulatory elements to an integrated splicing code. RNA (2008), 14:802–813

IAAM/IAAM diplotype is associated with extended ambulation in glucocorticoid-treated DMD patients





Genotype and tissue-specific gene expression from the GTEx Project



GTEx | Datasets | Gene Association | eQTL Browser | Sample Data | Documentation | Publications | Contact

Dataset Summary of Analysis Samples of the V6p Release

Data source: Release V6p (dbGaP Accession phs000424.v6.p1)

V6p Sample Info
- Sample Counts by Tissues
- eGenes vs Sample Size

V6p Donor Info
- Gender, Ethnicity & Age
- Cause of Death
- Tissue Counts Per Donor
- Age By Tissues

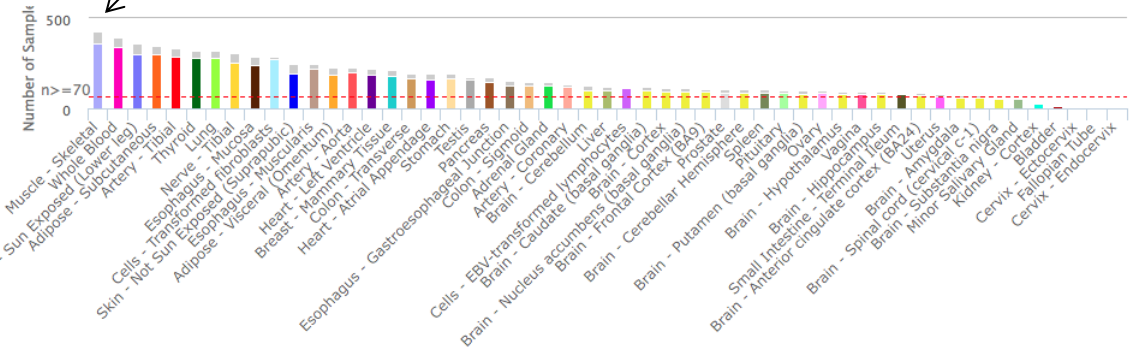
V6p Release	# Tissues	# Donors	# Samples
Total	53	544	8555
With Genotype	53	449	7333
Has eQTL Analysis*	44	449	7051

** Number of samples with genotype >= 70*

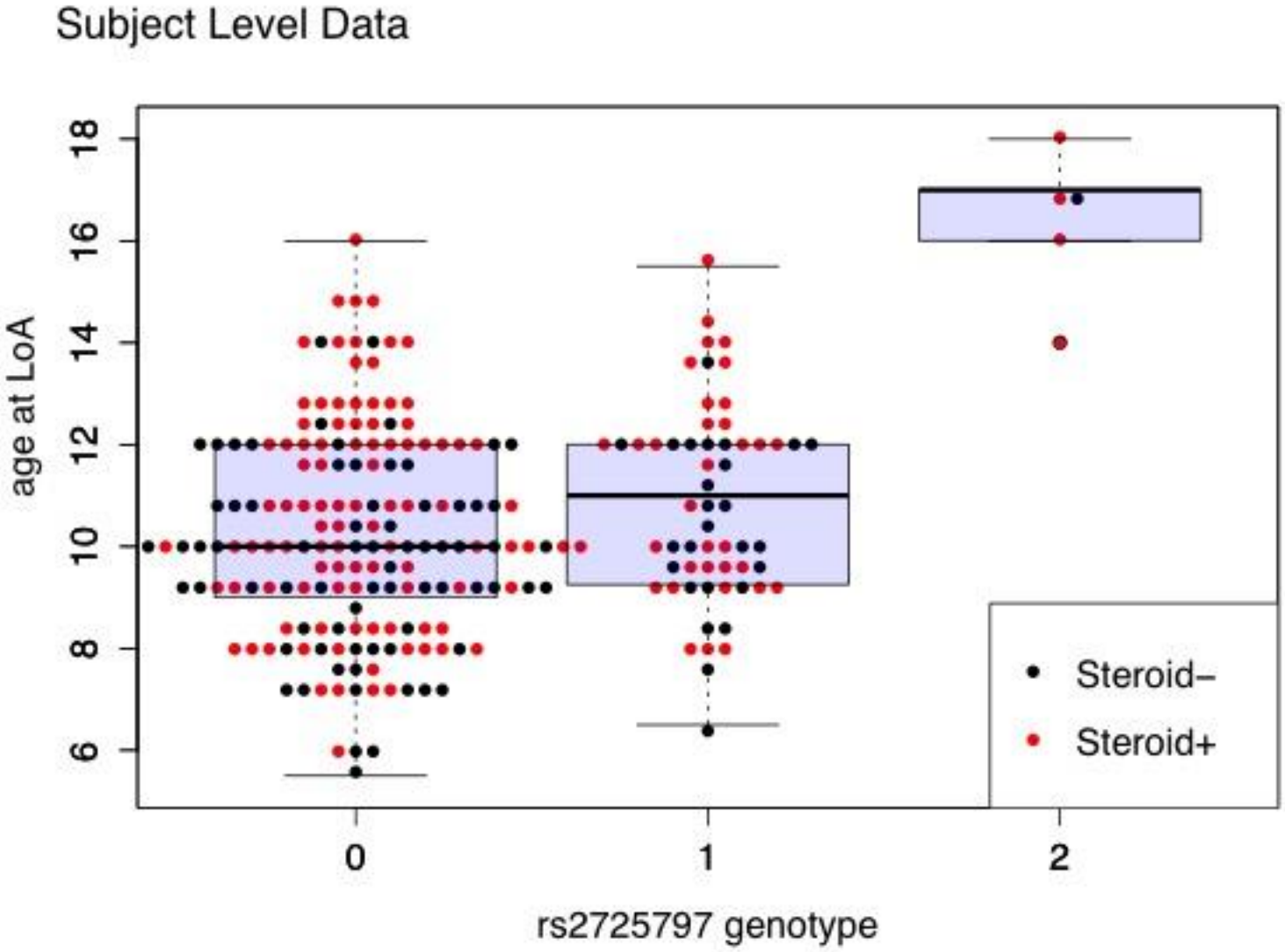
Skeletal muscle,
361 subjects with
genotype + RNA-Seq

V6p Sample Counts by Tissues

Sort tissues by: Count



The Genotype-Tissue Expression (GTEx) pilot analysis: Multitissue gene regulation in humans The GTEx Consortium. Science May 2015: Vol. 348, Issue 6235, pp. 648-660


Subject Level Data
age at LoA
6
8
10
12
14
16
18
0
1
2
rs2725797 genotype
Steroid−
Steroid+

LTBP4 Genotype Predicts Age of Ambulatory Loss in Duchenne Muscular Dystrophy

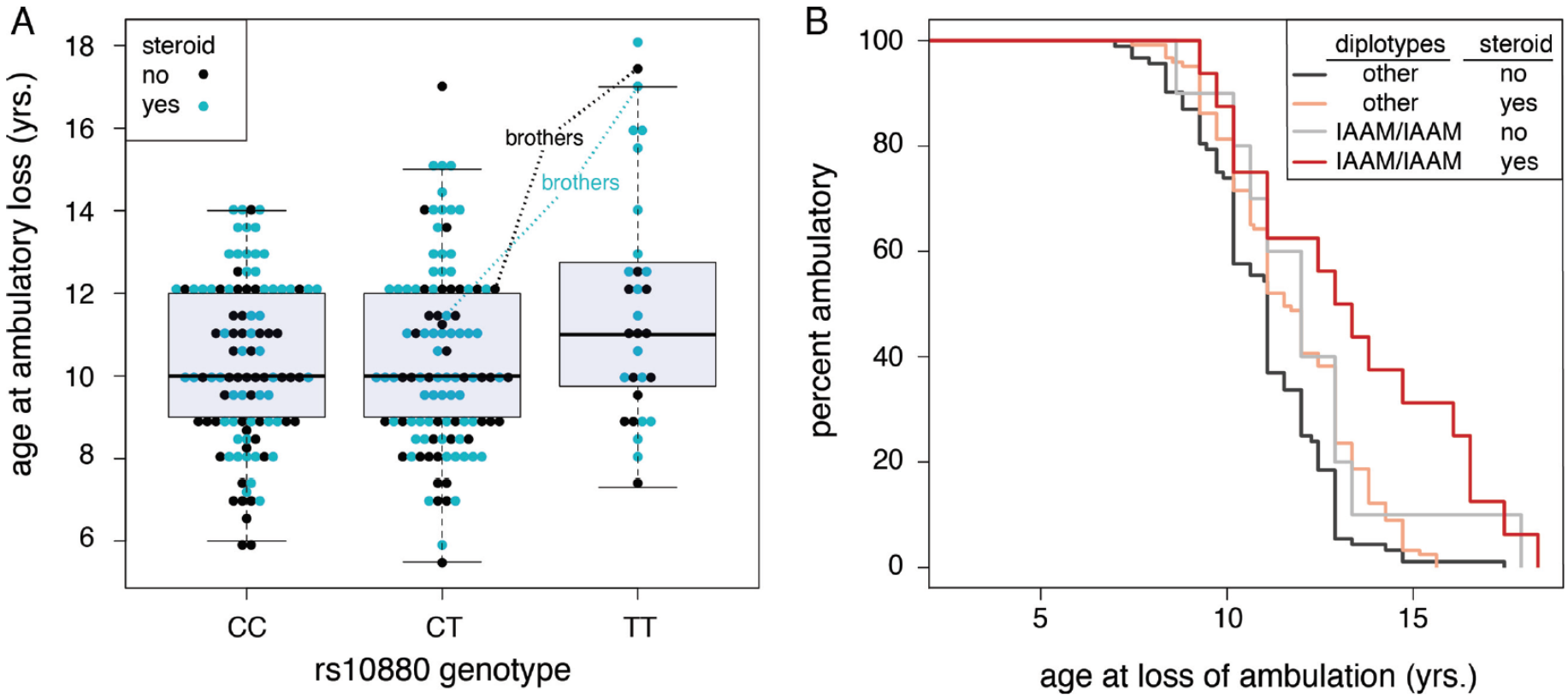


FIGURE 3: The *LTBP4* rs10880 minor homozygote (TT = Met:Met) and IAAM/IAAM diplotype is associated with extended ambulation in glucocorticoid-treated Duchenne muscular dystrophy (DMD) patients. (A) Age of ambulatory loss classified by rs10880 genotype and color-coded for patients by their steroid treatment. Two pairs of brothers discordant for their rs10880 genotypes are shown connected by the dotted lines. (B) Survival curves for DMD patients with the *LTBP4* IAAM/IAAM versus other haplotype pairs, with steroid-treated versus naive individuals plotted separately. The IAAM haplotype consists of single nucleotide polymorphisms rs2303729 (I = Ile), rs1131620 (A = Ala), rs1051303 (A = Ala), and rs10880 (M = Met) respectively. There were 16 IAAM homozygotes in the steroid treated group and 10 IAAM homozygotes in the naive group. [Color figure can be viewed in the online issue, which is available at www.annalsofneurology.org.]

DMD: loss of ambulation at less than age 12
IMD: loss of ambulation between ages 12 - 20
BMD: loss of ambulation at after age 20

Table 1. Summary of All Mutations Detected, by Class and Phenotype

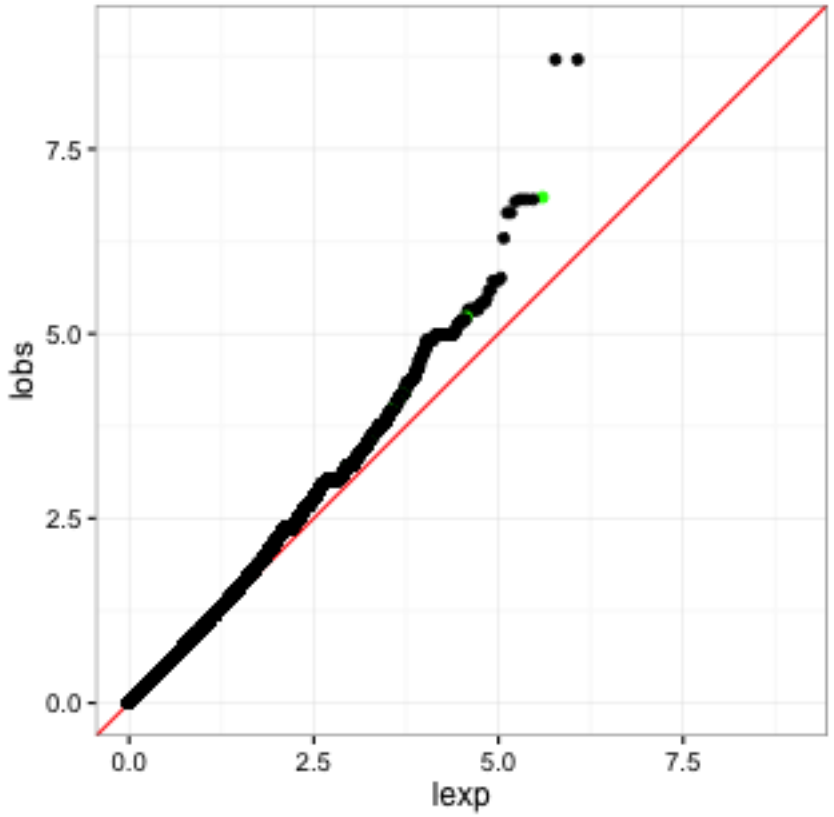
Mutation class	DMD	IMD	BMD	Unknown (B/DMD)	Manifesting carrier	Carrier (all phenotypes)	Total	%
Deletion	284	15	55	106	3	14	477	42.9%
in	*30*	*2*	*36*	*17*	*0*	*2*	***87***	
out	*244*	*13*	*18*	*88*	*2*	*12*	***376***	
other	*10*	*0*	*1*	*2*	*1*	*0*	***14***	
Stop	176	4	30	46	4	34	294	26.5%
UGA	*60*	*1*	*13*	*20*	*3*	*15*	***112***	
UAG	*71*	*0*	*11*	*13*	*0*	*4*	***99***	
UAA	*45*	*3*	*6*	*13*	*1*	*15*	***83***	
Subexonic	69	0	10	33	1	14	127	11.4%
FS Ins	*22*	*0*	*1*	*7*	*1*	*6*	***37***	
FS Del	*46*	*0*	*4*	*23*	*0*	*8*	***81***	
FS Ins/Del	*1*	*0*	*2*	*2*	*0*	*0*	***5***	
in-frame deletion	*0*	*0*	*3*	*1*	*0*	*0*	***4***	
Exonic duplication	87	7	10	8	5	5	122	11.0%
Splice	22	3	7	18	2	12	64	5.8%
Missense	2	1	6	6	0	0	15	1.4%
Pseudoexon	0	2	2	0	0	2	6	0.5%
Potential	2	0	0	3	0	1	6	0.5%
Other	0	0	0	0	0	0	0	0.0%
Total mutations	642	32	120	220	15	82	1,111	100.0%

"Carrier" are obligate or asymptomatic carriers, whereas "Manifesting Carriers" display symptoms of significant myalgia and/or weakness. (Further characterization of carrier phenotypic class is found in Supp. Table S2, listing specific mutations detected.)
DMD, Duchenne Muscular Dystrophy; IMD, intermediate muscular dystrophy; BMD, Becker Muscular Dystrophy; FS Ins, frameshift insertion; FD Del, frameshift deletion; FS Ins/Del, frameshift insertion/deletion.

HUMAN MUTATION, Vol. 30, No. 12, 1657–1666, 2009

TSP-1 is a major activator of latent TGF-β1

- Thrombospondin-1 (TSP-1, THBS1) binds small latent TGF-β and large latent TGF-β, and this binding interaction is sufficient to generate biologically active TGF-β.


7.5
5.0
2.5
0.0
lobs
0.0
2.5
5.0
7.5
lexp