Modifiers:
What changes DMD

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Disclosures

- Site principal investigator for PTC Therapeutics, Prosensa, Abeona Therapeutics, and the NIH FOR-DMD study; site co-investigator for Sarepta
- Advisory boards for Sarepta, PTC, Eli Lilly, 4D Therapeutics, and Dynacure
- Royalties from Audentes

Dystrophinopathies: Clinical diagnosis

DMD:

- Onset age 3-5
- Pelvic girdle weakness
- Tight heel cords
- CK 50-100X normal
- Loss of ambulation by age 12 (range 7-12)
- Mean age of death 19

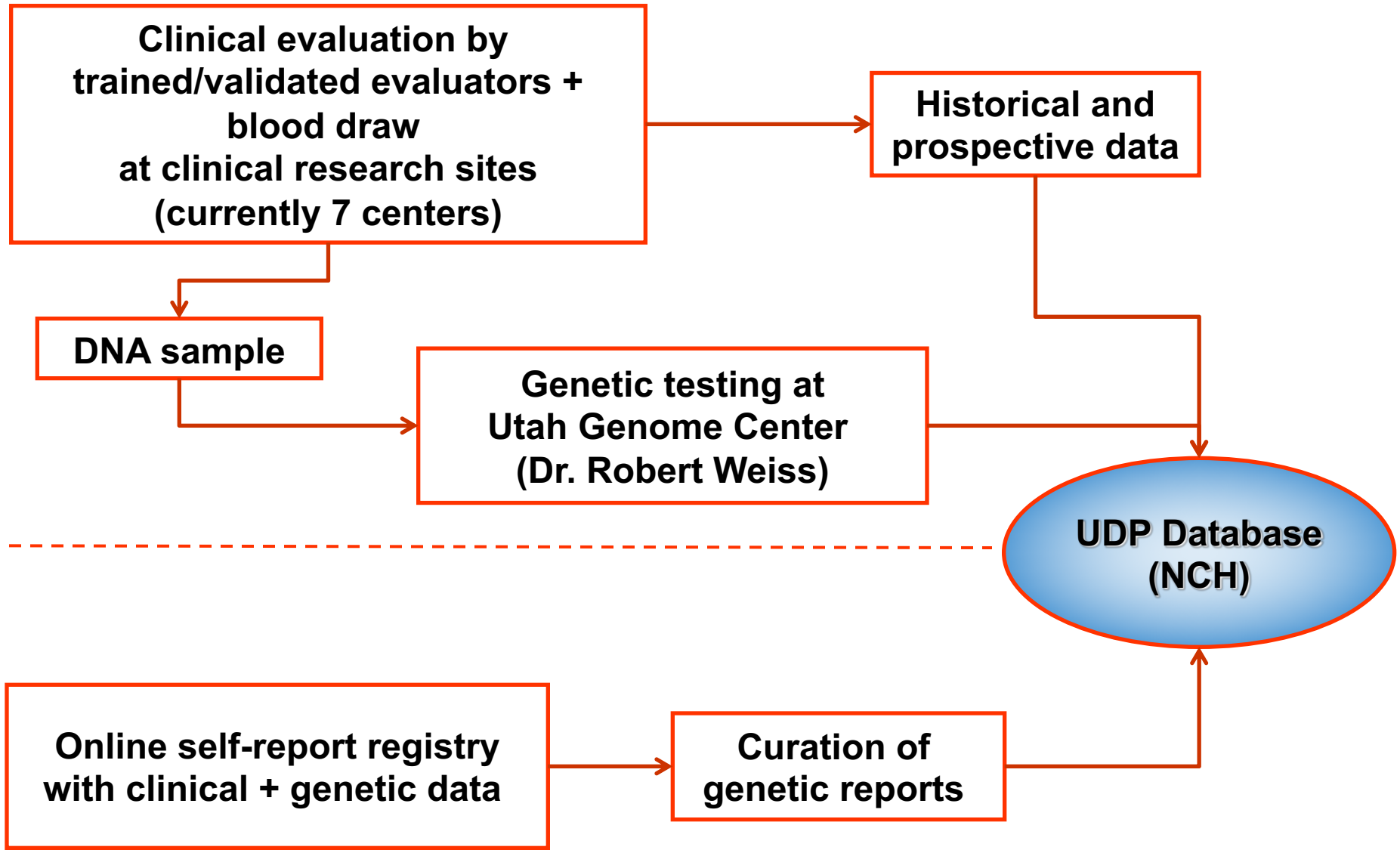
BMD:

- Classic definition: loss of ambulation > age 12
- Alternatively:
  - "intermediate muscular dystrophy" for loss of ambulation ages 12 through15
  - BMD for loss of ambulation >age 15
- Limb-girdle syndromes in adulthood
- Myalgias
- Isolated cardiomyopathy

Duchenne vs Becker: The “reading frame rule”

- Size of deletion does not correlate well with phenotype
- In-frame deletions are more likely to result in translation of a protein with partial function
- Out-of-frame deletions are DMD ~90% of the time
- *What accounts for patients who break the reading frame rule?*
- *What accounts for variability among patients with DMD?*

United Dystrophinopathy Project



UDP Genotype/Phenotype Database: enrollment diagnostic criteria

Clinical features of DMD/BMD and an X-linked family history

Or

Muscle biopsy demonstrating absent or altered dystrophin expression (immunofluorescence, immunohistochemistry, immunoblot)

Or

DMD gene mutation in previous clinical testing

RESEARCH ARTICLE

Human Mutation

OFFICIAL JOURNAL
HGVS
HUMAN GENOME VARIATION SOCIETY
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Mutational Spectrum of DMD Mutations in Dystrophinopathy Patients: Application of Modern Diagnostic Techniques to a Large Cohort

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Reading Frame Rule in DMD/BMD

Table 2. The Value of Mutational Reading Frame in Predicting a Phenotype of Duchenne Muscular Dystrophy

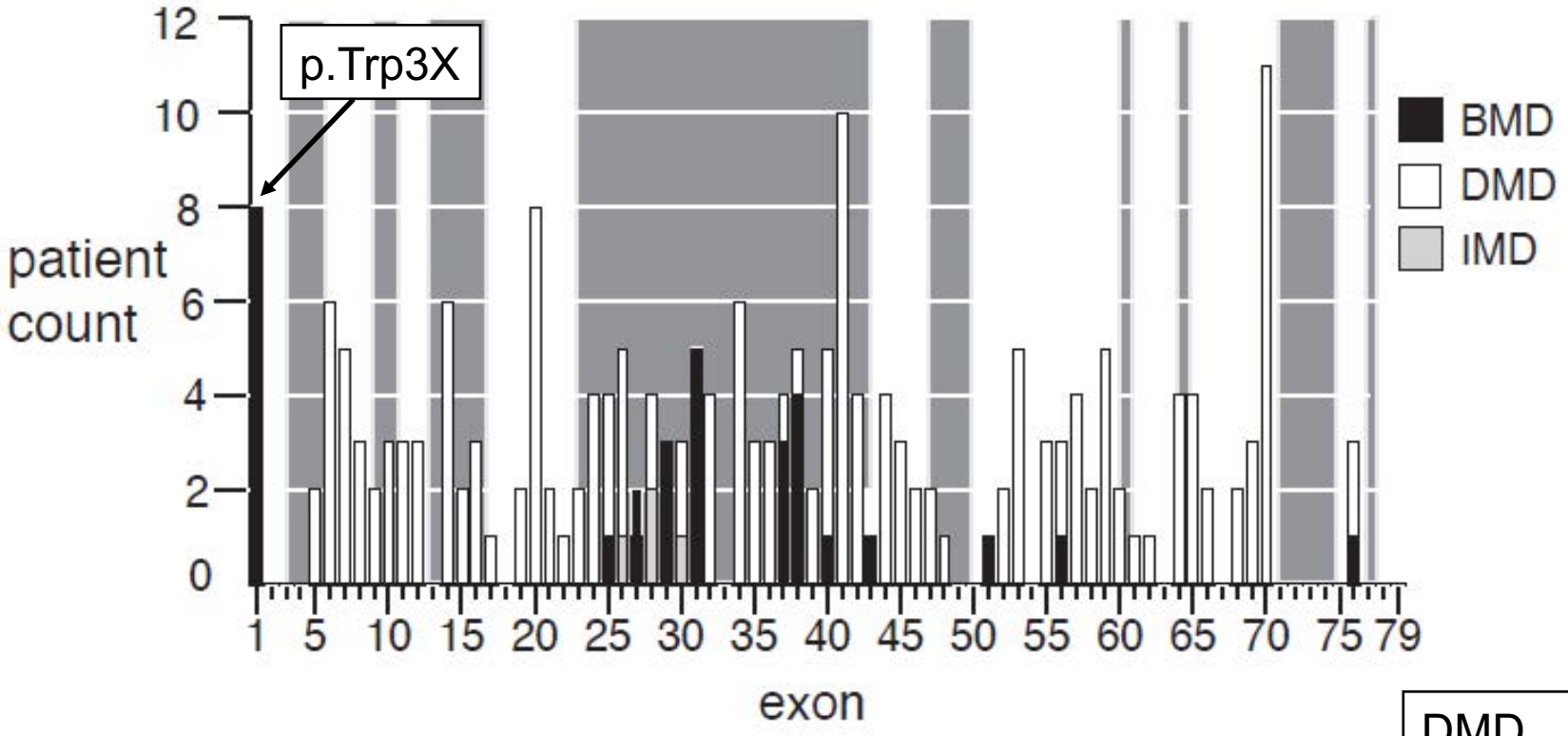
	DMD	I/BMD		
Exonic deletions only				
Truncating (out-of-frame) mutations	254	32	*88.8%*	*Positive predictive value*
Non-truncating (in-frame) mutations	30	38	*55.9%*	*Negative predictive value*
Sensitivity	89.4%	—		
Specificity	—	54.3%		
All mutations[a]				
Truncating mutations	519	79	*86.8%*	*Positive predictive value*
Non-truncating mutations	37	63	*63.0%*	*Negative predictive value*
Sensitivity	93.3%	—		
Specificity	—	44.4%		

45%-55% of BMD patients have out-of-frame mutations

Flanigan KM, et al. *Hum Mutat*. 2009;30(12):1657-1666.

Distribution of
BMD versus DMD nonsense mutations

In-frame exons (39) shaded Out-of-frame (40) unshaded

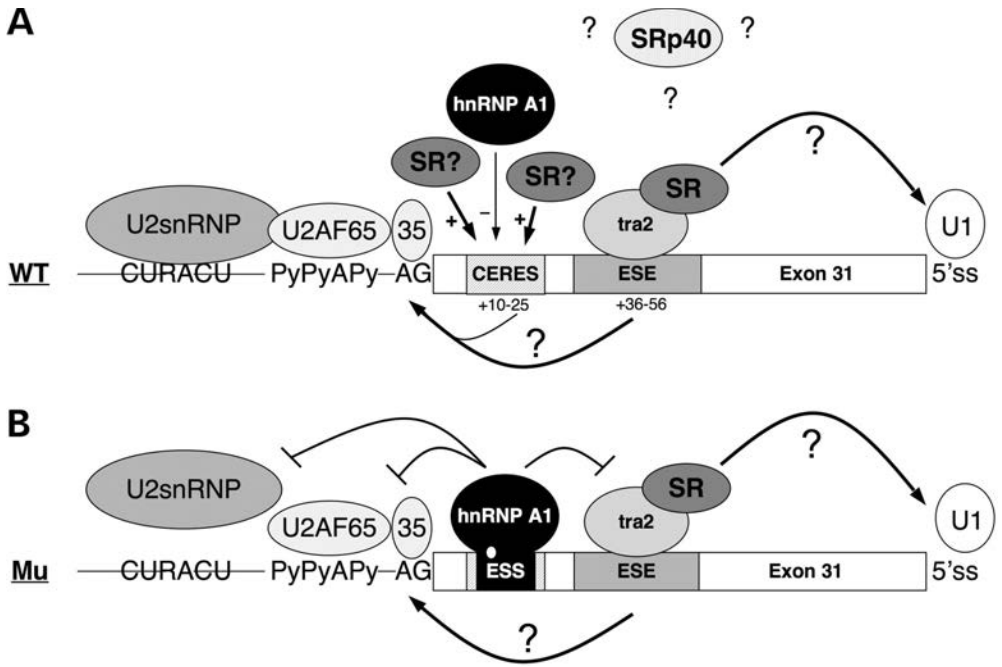


DMD	176
I/BMD	26

p = 0.004

Nonsense Mutations Do Not Always Predict DMD

- Mutations predicted as nonsense mutations may instead affect exon splice regulatory signals[1,2]
  - This results in exclusion of exons
  - The remaining mRNA may be in-frame



Disset A, et al. *Hum Mol Genet.* 2006;15(6);999-1013.

1. Disset A, et al. *Hum Mol Genet.* 2006;15(6);999-1013.
2. Flanigan KM, et al. *Hum Mutat*. 2011;32(3):299-308.

Modifiers of phenotype:

- Modifiers at the *DMD* locus:
  - Altered mRNA splicing (nonsense-associated BMD)
  - Altered protein translation initiation
    - p.Trp3X allele (N. American founder allele)

- Other genes that modify disease severity

SPP1 genotype is a determinant of disease severity in Duchenne muscular dystrophy

- Cytokine involved in immune cell migration and survival
- Implicated in fibrosis through the TGF-beta pathway

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ABSTRACT

Objective: Duchenne muscular dystrophy (DMD) is the most common single-gene lethal disorder. Substantial patient-patient variability in disease onset and progression and response to glucocorticoids is seen, suggesting genetic or environmental modifiers.

Methods: Two DMD cohorts were used as test and validation groups to define genetic modifiers: a Padova longitudinal cohort (n = 106) and the Cooperative International Neuromuscular Research Group (CINRG) cross-sectional natural history cohort (n = 156). Single nucleotide polymorphisms to be genotyped were selected from mRNA profiling in patients with severe vs mild DMD, and genome-wide association studies in metabolism and polymorphisms influencing muscle phenotypes in normal volunteers were studied.

Results: Effects on both disease progression and response to glucocorticoids were observed with polymorphism rs28357094 in the gene promoter of SPP1 (osteopontin). The G allele (dominant model; 35% of subjects) was associated with more rapid progression (Padova cohort log rank $p = 0.003$), and 12%–19% less grip strength (CINRG cohort $p = 0.0003$).

Conclusions: Osteopontin genotype is a genetic modifier of disease severity in Duchenne dystrophy. Inclusion of genotype data as a covariate or in inclusion criteria in DMD clinical trials would reduce intersubject variance, and increase sensitivity of the trials, particularly in older subjects.

Neurology® 2011;76:219–226

Using mice to map genetic modifiers

Beth McNally, MD/PhD (Northwestern)

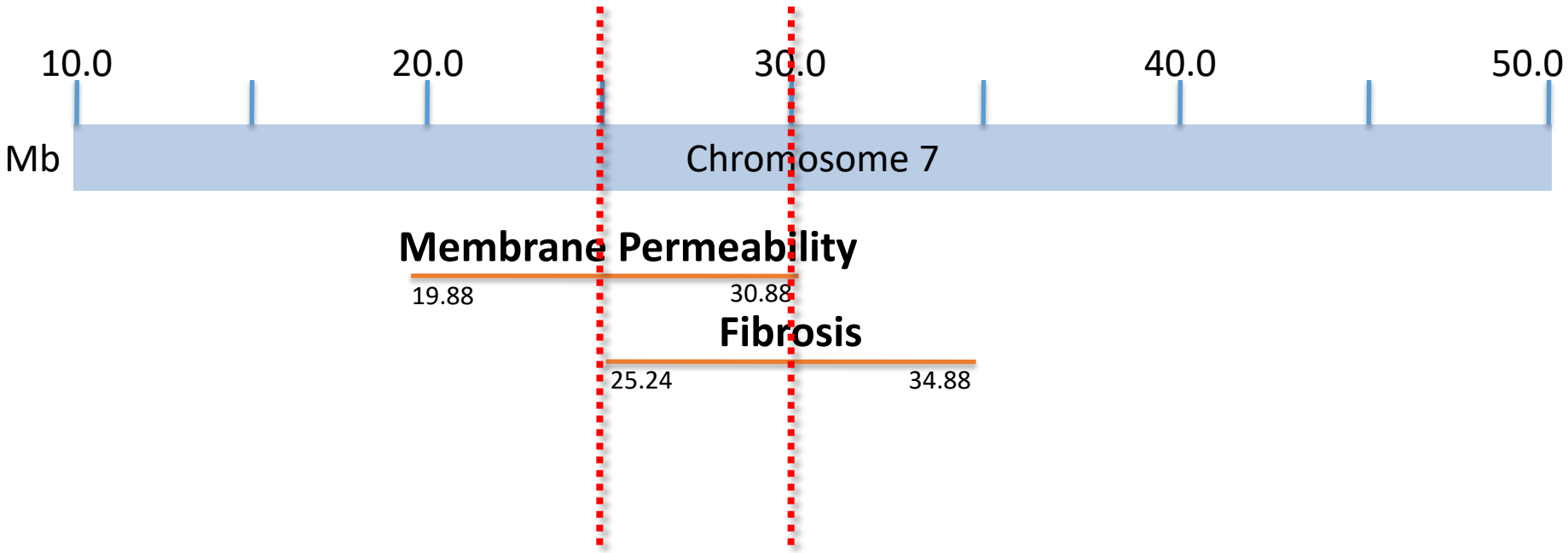
Parental strains: ***Sgcg*^{D2} (severe)** **X** ***Sgcg*^{129} (mild)**

F1 generation: ***Sgcg*$^{D2/129}$**

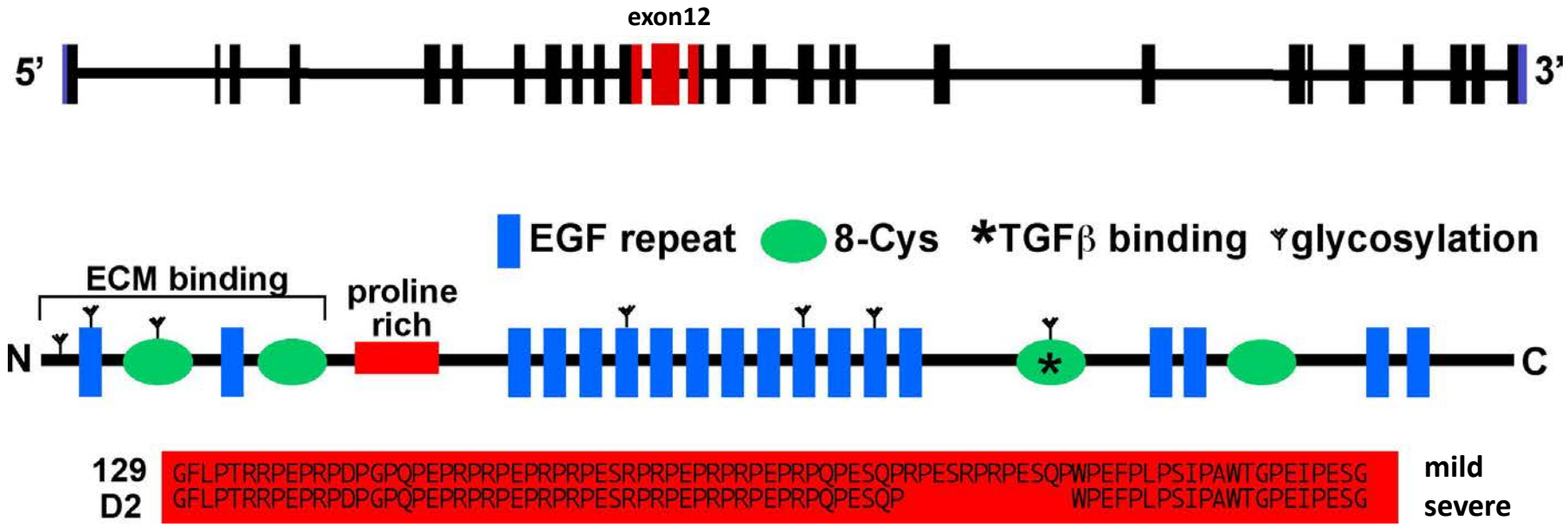
F2 generation ***Sgcg*$^{D2/129}$**

- **270 F2 animals were analyzed.**

Membrane permeability and fibrosis are both modified by a region of chr 7

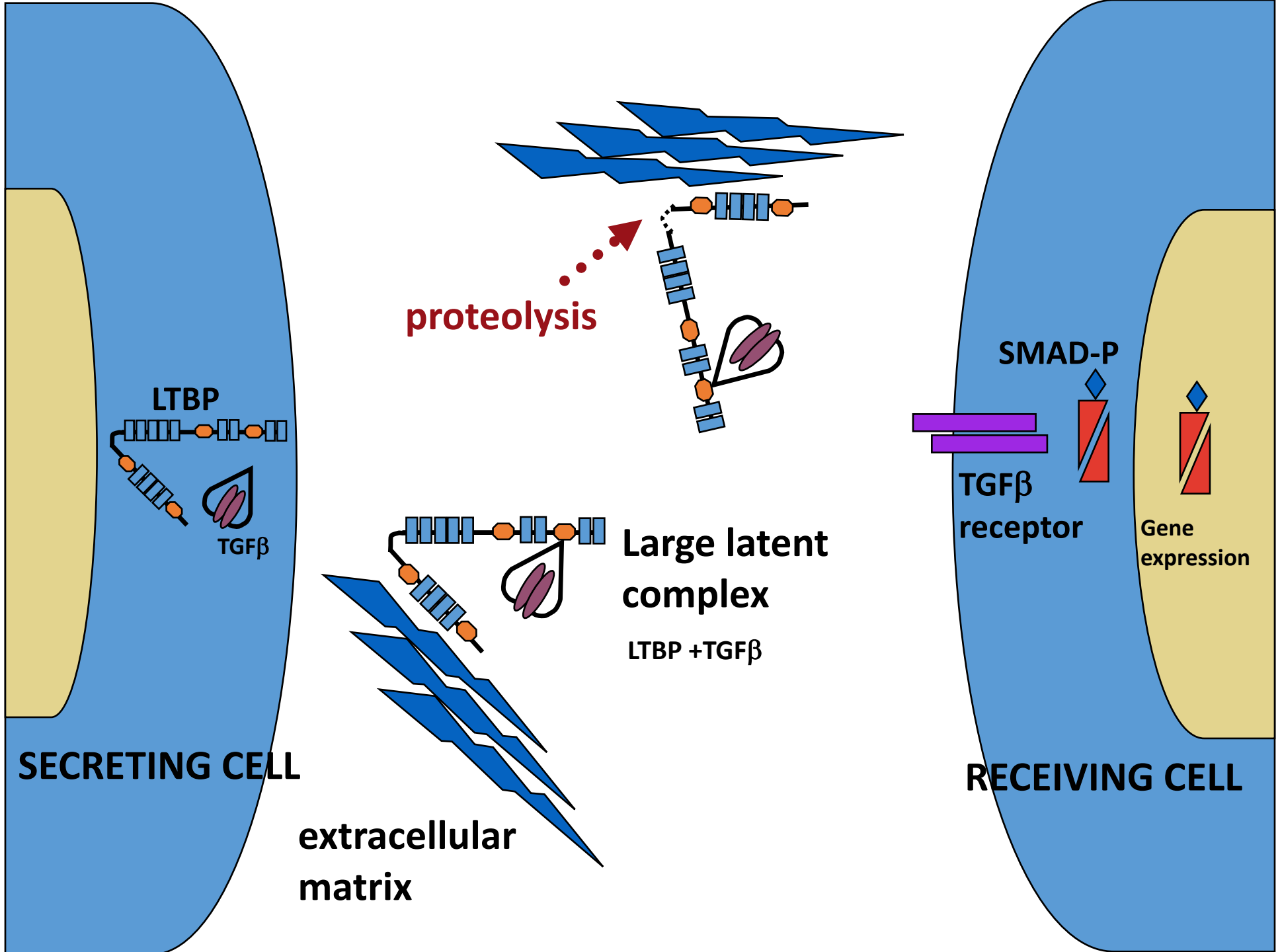


An insertion/deletion in LTBP4 modifies muscular dystrophy



exon12

Heydemann et al. 2009


LTBP
TGFβ
SECRETING CELL
proteolysis
Large latent complex
LTBP +TGFβ
extracellular matrix
SMAD-P
TGFβ receptor
Gene expression
RECEIVING CELL

Does *LTBP4* influence DMD phenotype?

- Use loss of ambulation as a dichotomous outcome
- To take an unbiased approach, we included all subjects who had a recorded loss of ambulation before age 20, regardless of what phenotype was recorded
- N = 254 subjects
  - 244 (96%) were catalogued as DMD
  - 8 = IMD (lost ambulation between age 12 and 15)
  - 2 = BMD (lost ambulation after age 15 but before age 20)

LTBP4 Genotype Predicts Age of Ambulatory Loss in Duchenne Muscular Dystrophy

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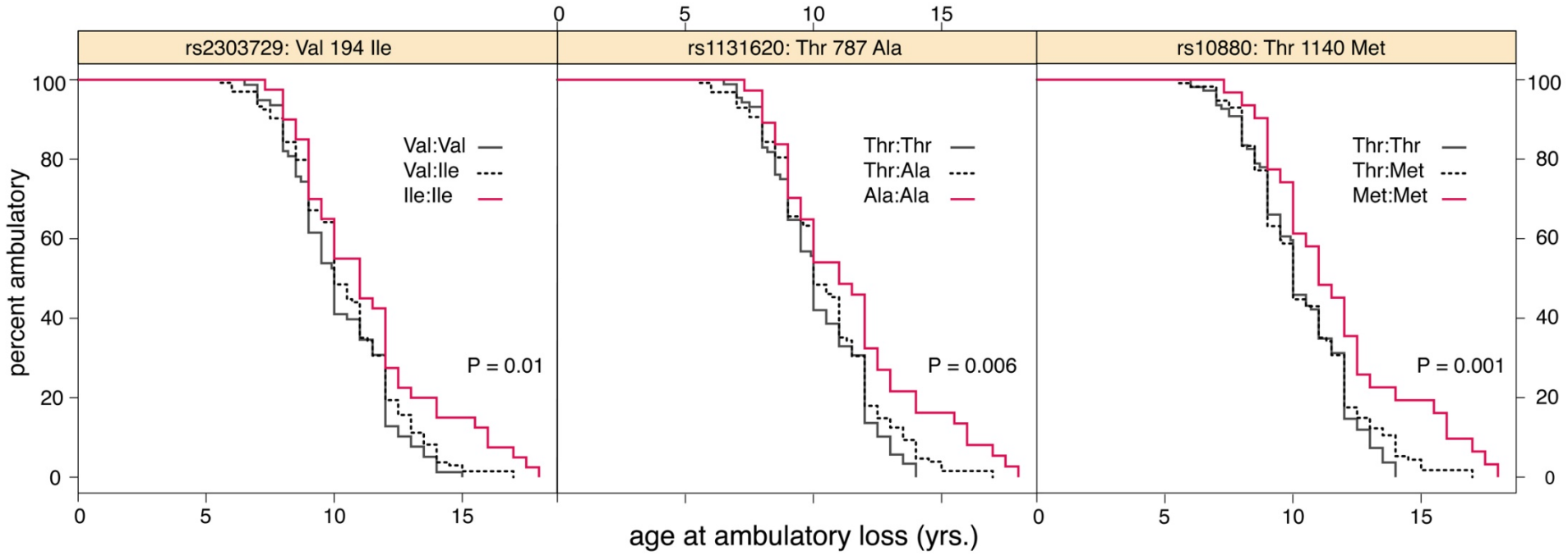
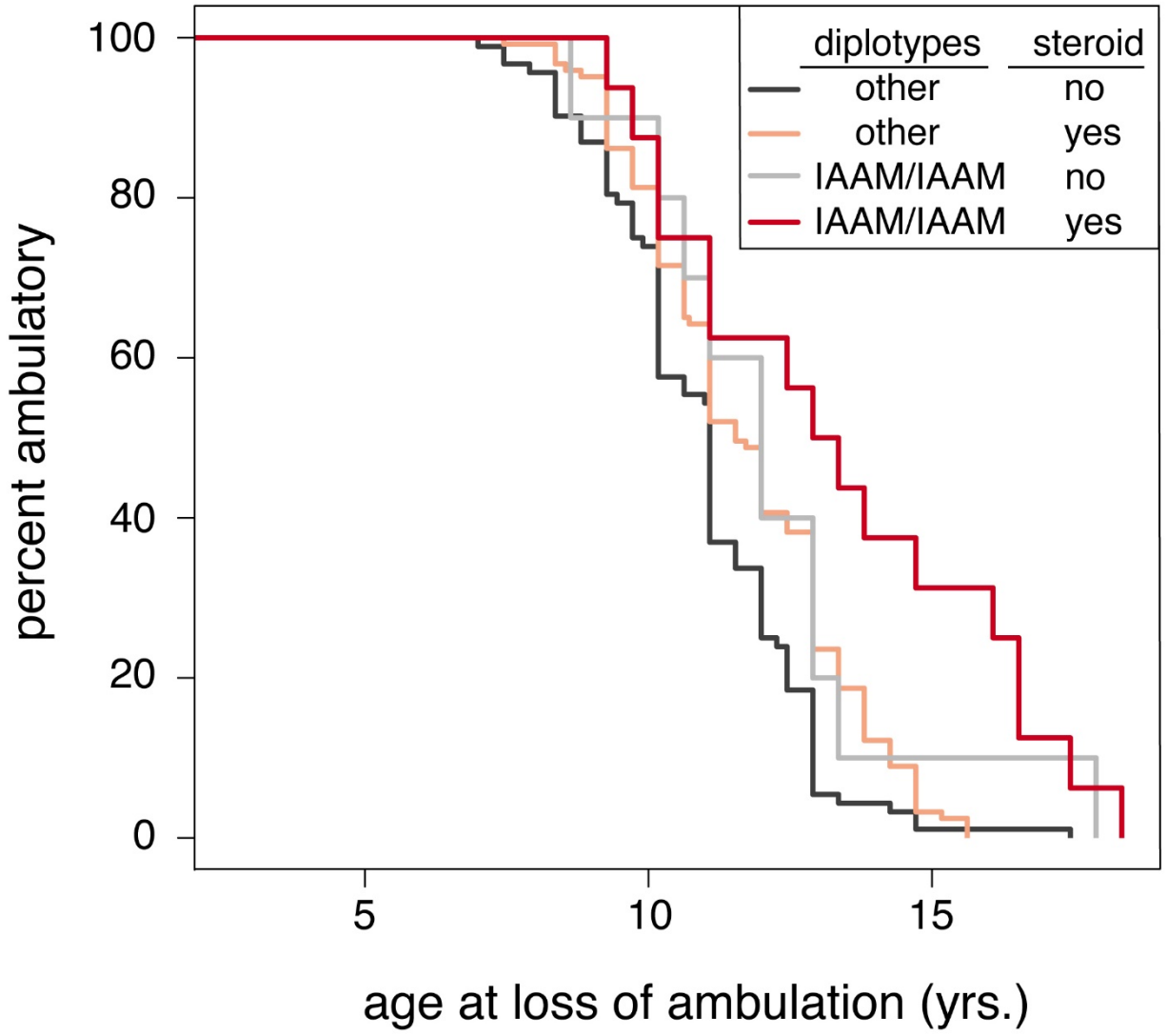
Objective: Duchenne muscular dystrophy (DMD) displays a clinical range that is not fully explained by the primary *DMD* mutations. *Ltbp4*, encoding latent transforming growth factor-β binding protein 4, was previously discovered in a genome-wide scan as a modifier of murine muscular dystrophy. We sought to determine whether *LTBP4* genotype influenced DMD severity in a large patient cohort.
Methods: We analyzed nonsynonymous single nucleotide polymorphisms (SNPs) from human *LTBP4* in 254 nonambulatory subjects with known *DMD* mutations. These SNPs, V194I, T787A, T820A, and T1140M, form the VTTT and IAAM *LTBP4* haplotypes.
Results: Individuals homozygous for the IAAM *LTBP4* haplotype remained ambulatory significantly longer than those heterozygous or homozygous for the VTTT haplotype. Glucocorticoid-treated patients who were IAAM homozygotes lost ambulation at 12.5 $\pm$ 3.3 years compared to 10.7 $\pm$ 2.1 years for treated VTTT heterozygotes or homozygotes. IAAM fibroblasts exposed to transforming growth factor (TGF) β displayed reduced phospho-SMAD signaling compared to VTTT fibroblasts, consistent with LTBP4's role as a regulator of TGFβ.
Interpretation: *LTBP4* haplotype influences age at loss of ambulation, and should be considered in the management of DMD patients.

ANN NEUROL 2013;73:481–488

The *LTBP4* "IAAM" haplotype predicts prolonged ambulation in DMD



Steroid use with *LTBP4* "IAAM" predicts prolonged ambulation in DMD



Haplotype analysis of nonsynonymous *LTBP4* variants associated with age of ambulatory loss

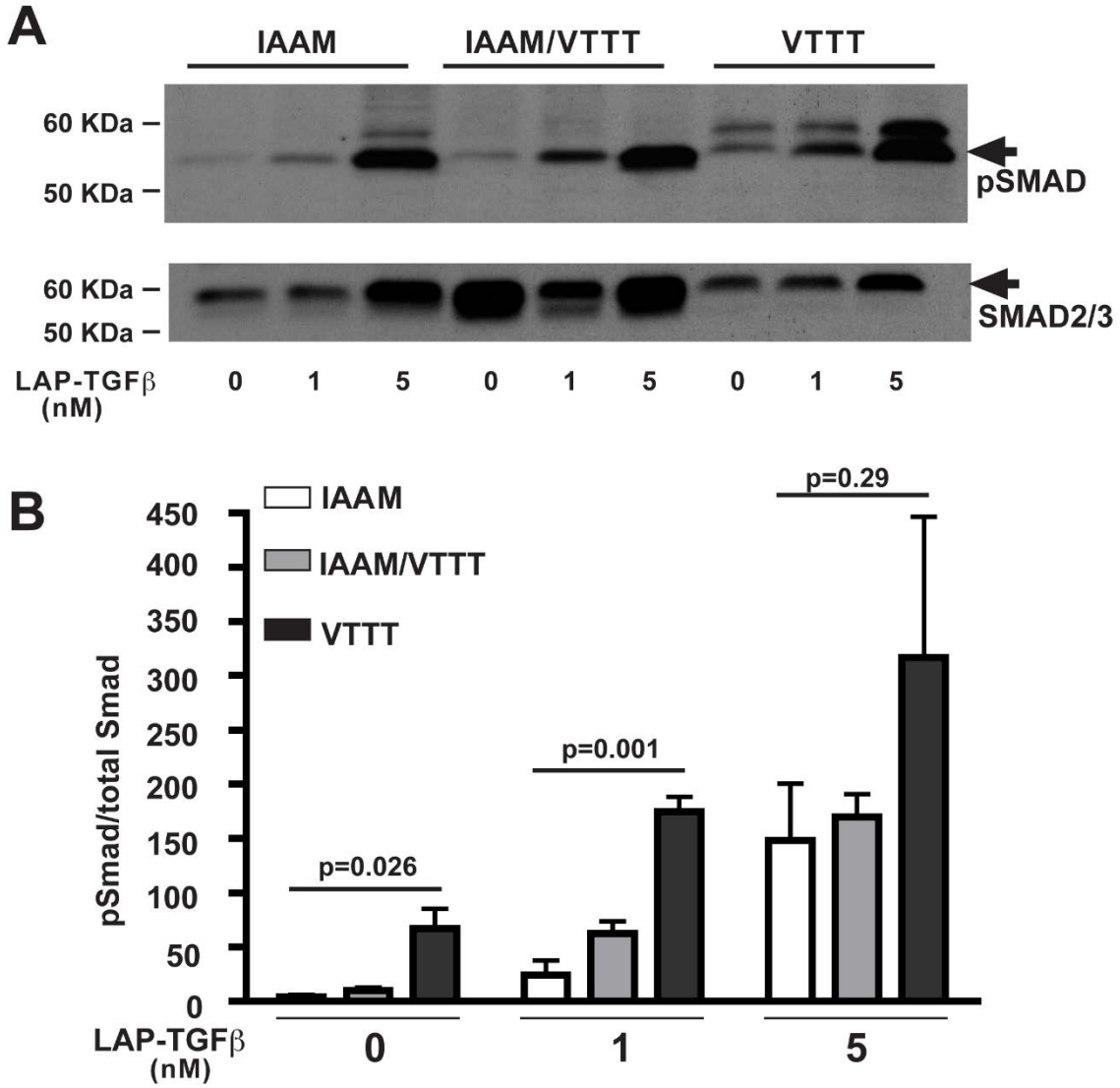
	All (n=254) Global P[a] = 0.002			Steroid treated (n=137) Global P[a] = 0.013			Steroid naive (n=102) Global P[a] = 0.12		
Haplotype[b]	freq	score	p-val[c]	freq	score	p-val[c]	freq	score	p-val[c]
VTTT	0.53	-1.51	0.1	0.52	-1.04	0.3	0.52	-1.12	0.3
IAAM	0.31	3.43	6×10^{-4}	0.32	2.92	0.004	0.29	1.91	0.06

[a] 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.
[b] These haplotypes consist of SNPS: rs2303729, rs1131620, rs10880 respectively.
[c] p value from the $\chi 2$, df=1, distribution of the haplotype-specific score.

Mean age of ambulatory loss for "IAAM" versus other haplotypes:

- In steroid-treated patients: 12.5 ± 3.3 years vs. 10.7 ± 2.1 years
- In steroid-naïve patients: 11.2 ± 2.7 vs. 9.8 ± 2.0 years

LTBP4 "IAAM" fibroblasts have reduced TGFβ signaling



Our next step:
Genome-wide association studies

- Instead of looking at a candidate gene (like *LTBP4*), what if we look at 2.5 million variants (SNPs, or single nucleotide polymorphisms) throughout the whole genome?

- Affymetrix OmniChip
- Same 254 subjects

Long-Range Genomic Regulators of *THBS1* and *LTBP4* Modify Disease Severity in Duchenne Muscular Dystrophy

Robert B. Weiss, PhD,[1] Veronica J. Vieland, PhD,[2,4,6] Diane M. Dunn, BS,[1]
Yuuki Kaminoh, BS,[3] and Kevin M. Flanigan, MD,[3,4,5]
for the United Dystrophinopathy Project

Objective: Duchenne muscular dystrophy (DMD) is a severe X-linked recessive disease caused by loss-of-function dystrophin (*DMD*) mutations in boys, who typically suffer loss of ambulation by age 12. Previously, we reported that coding variants in latent transforming growth factor beta (TGFβ)-binding protein 4 (*LTBP4*) were associated with reduced TGFβ signaling and prolonged ambulation ($p = 1.0 \times 10^{-3}$) in DMD patients; this result was subsequently replicated by other groups. In this study, we evaluated whether additional DMD modifier genes are observed using whole-genome association in the original cohort.

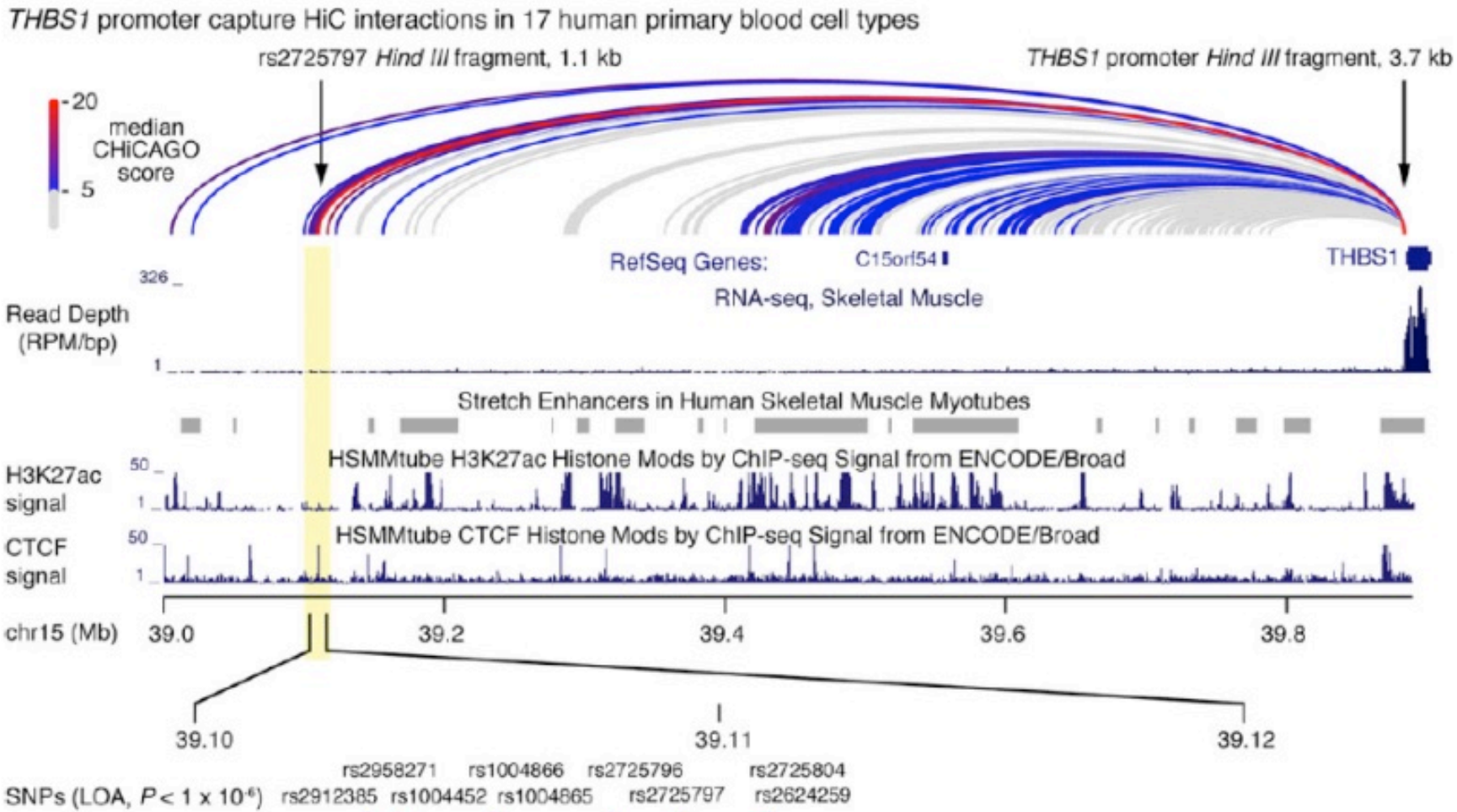
Methods: We performed a genome-wide association study (GWAS) for single-nucleotide polymorphisms (SNPs) influencing loss of ambulation (LOA) in the same cohort of 253 DMD patients used to detect the candidate association with *LTBP4* coding variants. Gene expression and chromatin interaction databases were used to fine-map association signals above the threshold for genome-wide significance.

Results: Despite the small sample size, two loci associated with prolonged ambulation met genome-wide significance and were tagged by rs2725797 (chr15, $p = 6.6 \times 10^{-9}$) and rs710160 (chr19, $p = 4.7 \times 10^{-8}$). Gene expression and chromatin interaction data indicated that the latter SNP tags regulatory variants of *LTBP4*, whereas the former SNP tags regulatory variants of thrombospondin-1 (*THBS1*): an activator of TGFβ signaling by direct binding to LTBP4 and an inhibitor of proangiogenic nitric oxide signaling.

Interpretation: Together with previous evidence implicating *LTBP4*, the *THBS1* modifier locus emphasizes the role that common regulatory variants in gene interaction networks can play in mitigating disease progression in muscular dystrophy.

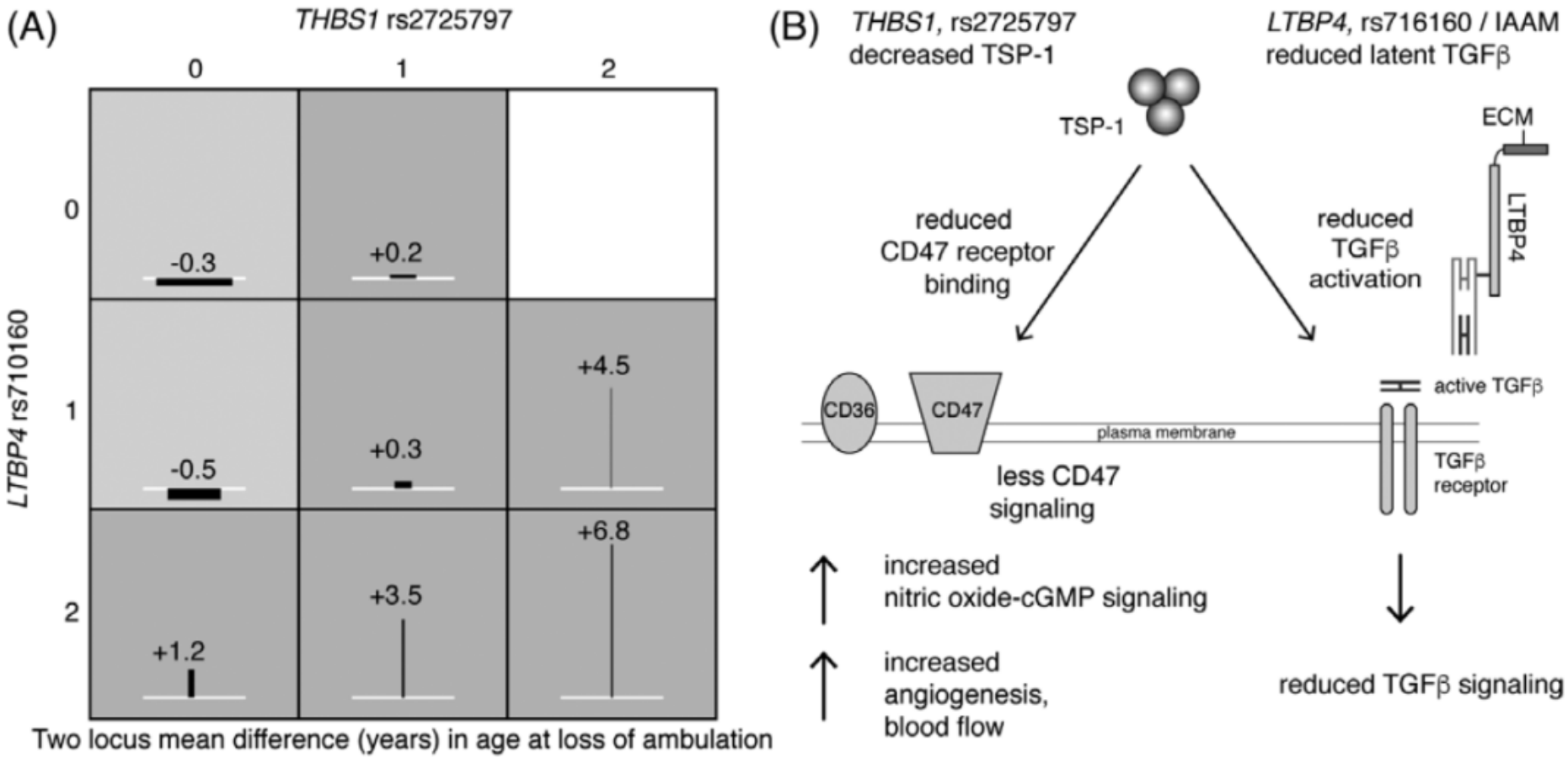
ANN NEUROL 2018;84:234–245

SNPs found in a gene desert upstream from *THBS1* locus



THBS1

- Encodes encodes thrombospondin-1 (TSP-1)
- Multifunctional, extracellular matrix (ECM) glycoprotein that is a major activator of TGFβ signaling
  - Directly interacts with LTBP4 in the ECM.
- Also an anti-angiogenic factor
- THBS1 expression is elevated in *mdx* mice


(A)
THBS1 rs2725797
0
1
2
LTBP4 rs710160
0
1
2
-0.3
+0.2
-0.5
+0.3
+4.5
+1.2
+3.5
+6.8
Two locus mean difference (years) in age at loss of ambulation
(B)
THBS1, rs2725797
decreased TSP-1
LTBP4, rs716160 / IAAM
reduced latent TGFβ
TSP-1
ECM
LTBP4
reduced
CD47 receptor
binding
reduced
TGFβ
activation
CD36
CD47
plasma membrane
active TGFβ
TGFβ
receptor
less CD47
signaling
increased
nitric oxide-cGMP signaling
increased
angiogenesis,
blood flow
reduced TGFβ signaling

Conclusions

- The TGFβ signaling pathway is a point of convergence for modifiers of disease severity

- The *LTBP4* IAAM haplotype is associated with dereased TGFβ signaling.
  - seen independent of the primary mutation (truncating or not)
  - seen in both glucocorticoid treated and naïve DMD subjects

- This effect is amplified by the protective THBS1 allele.

- These modifiers should be considered in interpreting the results of clinical trials.

Ongoing search for new modifiers

- NIH (NINDS) project
- We are re-contacting families who participated in the United Dystrophinopathy Project to determine clinical outcomes, including
  - Age at wheelchair use
  - Age at diagnosis of cardiomyopathy
  - Age of BiPAP use
  - Survival
- Studying 2.5 million genetic locations (SNPs) within the genome for evidence of an association with better or worse prognosis.
- Exome sequencing in a subset of patients
- If you previously enrolled in the UDP, you may receive a call!

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