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Association Study of Exon Variants in the NF-κB and TGFβ Pathways Identifies *CD40* as a Modifier of Duchenne Muscular Dystrophy

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The expressivity of Mendelian diseases can be influenced by factors independent from the pathogenic mutation: in Duchenne muscular dystrophy (DMD), for instance, age at loss of ambulation (LoA) varies between individuals whose *DMD* mutations all abolish dystrophin expression. This suggests the existence of *trans*-acting variants in modifier genes. Common single nucleotide polymorphisms (SNPs) in candidate genes (*SPP1*, encoding osteopontin, and *LTBP4*, encoding latent transforming growth factor β [TGFβ]-binding protein 4) have been established as DMD modifiers. We performed a genome-wide association study of age at LoA in a sub-cohort of European or European American ancestry (n = 109) from the Cooperative International Research Group Duchenne Natural History Study (CINRG-DNHS). We focused on protein-altering variants (Exome Chip) and included glucocorticoid treatment as a covariate. As expected, due to the small population size, no SNPs displayed an exome-wide significant *p* value ($< 1.8 \times 10^{-6}$). Subsequently, we prioritized 438 SNPs in the vicinities of 384 genes implicated in DMD-related pathways, i.e., the nuclear-factor-κB and TGFβ pathways. The minor allele at rs1883832, in the 5′-untranslated region of *CD40*, was associated with earlier LoA ($p = 3.5 \times 10^{-5}$). This allele diminishes the expression of CD40, a co-stimulatory molecule for T cell polarization. We validated this association in multiple independent DMD cohorts (United Dystrophinopathy Project, Bio-NMD, and Padova, total n = 660), establishing this locus as a DMD modifier. This finding points to cell-mediated immunity as a relevant pathogenetic mechanism and potential therapeutic target in DMD.

Duchenne muscular dystrophy (DMD [MIM: 310200]), one of the most common lethal genetic disorders, is caused by frameshifting or truncating mutations in *DMD* (MIM: 300377), which encodes the protein dystrophin.[1] Despite dystrophin's being absent, or no more abundant than trace quantities, in the muscle fibers of all individuals living with DMD,[2] substantial variability is observed in clinical severity. For instance, age at loss of independent ambulation (LoA) may vary by several years.[3] Age at LoA is a clinically meaningful measure of disease severity in DMD and is correlated with respiratory failure, need for spinal surgery, and survival.[4] Understanding the bases of its variability could point at novel therapeutic targets, improve prognosis and personalization of treatments, and allow more accurate design and analysis of clinical trials.

Proof of principle that DMD severity may be modulated by *trans*-acting variants in genes different from *DMD* has been provided by association studies in *SPP1*[5–7] (MIM: 166490) and *LTBP4*[7–9] (MIM: 604710). These genes had emerged as candidate modifiers either from gene expression studies in samples of muscle tissue from severely versus mildly affected individuals with DMD (*SPP1*[5]) or from genome scans in murine models of muscular dystrophy (*LTBP4*[10]). Described variants have a regulatory function[11] or alter protein sequence,[8] and they delay median LoA by approximately 1 to 2 years.[5–9] If DMD severity is regarded as a complex trait, such a large effect might seem surprising for common variants (minor-allele frequency [MAF] > 0.05). However, coding or regulatory variants might acquire a strong modifier function, despite their high MAF in the general healthy population, if corresponding genes are upregulated under pathological conditions in a rare Mendelian disease.

LoA data in the Cooperative International Neuromuscular Research Group Duchenne Natural History Study (CINRG-DNHS,[12,13] NCT00468832) have been instrumental to the

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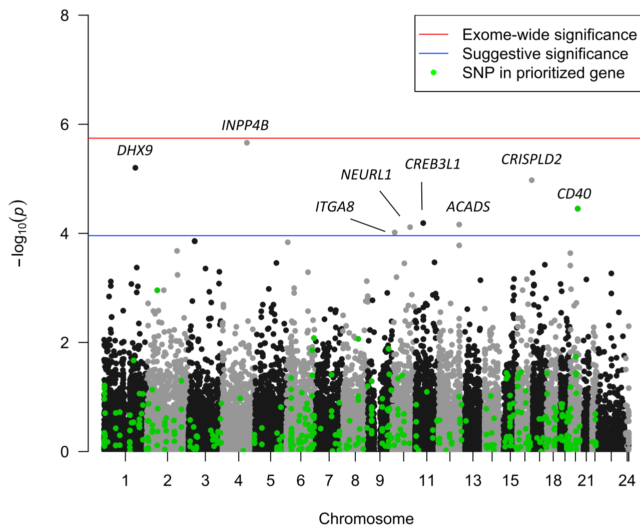

Figure 1. Manhattan Plot of Exome Chip Associations with Age at Loss of Ambulation

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 or downstream of, prioritized genes in the NF-κB and TGFβ pathways are highlighted in green. The red horizontal line ($p = 1.8 \times 10^{-6}$) refers to Bonferroni correction for 27,025 SNPs, and the blue line ($p = 1.1 \times 10^{-4}$) refers to Bonferroni correction for 438 SNPs within prioritized genes. Top p values are annotated with corresponding gene names (also see Table 1).

study of known genetic modifiers,[6,7] clinical variability associated with specific mutations,[14] and long-term effects of glucocorticoid corticosteroid (GC) treatment.[14,15] We aimed to discover genetic associations with age at LoA in this DMD cohort by genotyping participants with the Exome Chip. This chip is focused on functional (coding or regulatory) variants within or close to gene-coding regions. Intronic or intergenic single-nucleotide polymorphisms (SNPs) are also included, mainly on the basis of previous hits in a genome-wide association study (GWAS) or other evidence of a functional regulatory role. Details of chip design are publicly available (see Web Resources).

All participants included in this study and/or their legal guardians consented specifically to genotyping of genetic variants (SNPs) for research purposes, and the study was approved by local IRBs. Exome-chip genotyping and data-cleaning methods in the CINRG-DNHS cohorts have been previously described.[7] In brief, genotyping with the Illumina (San Diego, CA) HumanExome chip was performed in 175/340 CINRG-DNHS participants of different ethnicities; these individuals were selected on the basis of sufficient quantity and quality of available DNA and did not differ from the whole cohort in terms of clinical or demographic features. Genotype calling was performed with Genome Studio software, and genotype data were exported into PLINK format with the dedicated plug-in software by Illumina. Data cleaning was performed by PLINK[16] and included the following: missing-call thresholds of 0.01 for both samples and SNP assays; a heterozygosity threshold of ±4 standard deviations from the mean; and a check for cryptic duplicates and relatedness in an IBS matrix (PIHAT threshold of 0.1). A subcohort of 109 unrelated individuals of European or European American descent was selected by multidimensional scaling (MDS) analysis of exome-chip genotypes as described,[7] and repeated MDS showed no relevant population stratification in the selected subcohort (Figure S1 in the Supplemental Data available with this article online).

Genome-wide association with age at LoA was tested in these 109 participants ("Exome Chip cohort") with a Cox proportional hazards model. The dependent variable (phenotype) was age at LoA, and participants who were ambulatory at the last evaluation were censored. The independent variables were genotypes (additive inheritance model) at 27,025 Exome Chip SNPs with MAF > 0.05 and GC treatment coded as a binary categorical covariate, i.e., treatment for at least 1 year while the participant was ambulatory versus treatment < 1 year or no treatment while the participant was ambulatory. The MAF threshold was adopted because a small population size precluded single-SNP or groupwise rare-variant analyses. We performed the Cox proportional hazards test by plugging the R function "coxph" into PLINK via the Rserve package. A Bonferroni corrected p value of 1.8×10^{-6} (0.05/27,025 SNPs, "exome-wide" significance) was set for this analysis. R v. 3.2.1 was used for statistical analyses and graphical representation. QQ and Manhattan plots were created with the "qqman" package[17] in R.

The quantile-quantile (QQ) plot of observed p values (Figure S2) excluded major systematic bias ($\lambda_{GC} = 1.09$). No SNPs reached the "exome-wide" significance threshold of $p < 1.8*10^{-6}$ (Bonferroni correction for 27,025 SNPs, Manhattan plot shown in Figure 1). Top *P*-value annotations are shown in Table 1.

Due to acknowledged low statistical power in the initial GWAS, lack of "exome-wide" significance could be expected. Nevertheless, some nominally significant p values may indicate true associations. Therefore, we proceeded to prioritizing SNPs for validation in independent cohorts. To this end, we hypothesized that SNPs lying within, or less than 10 Kb upstream or downstream of genes involved in the nuclear factor κB (NF-κB) and transforming growth factor β (TGFβ) signaling pathways would be enriched for true associations. These are extensively studied inflammatory and pro-fibrotic pathways, implicated in relevant pathological events downstream of dystrophin deficiency.[18–21] Furthermore, both known DMD modifiers, derived from unbiased hypothesis-generating experiments (expression profiles for *SPP1*,[5] murine genome mapping for *LTBP4*[8]), are involved in these pathways.[5,19,22–24]

We selected 384 genes annotated as "I-κB kinase/NF-κB signaling" ([Gene Ontology] GO: 0007249), and/or "TGFβ receptor signaling pathway" (GO: 0007179). Gene names are reported in Table S1. Subsequently, we prioritized SNPs in the genomic regions corresponding to, or within 10 Kb upstream or downstream of, these genes.

Table 1. SNPs Showing Top p Values in the GWAS of Age at Loss of Ambulation in 109 Unrelated Participants of European Ancestry in the CINRG-DNHS

SNP	Chr	BP	Alleles	Minor Allele	MAF	BP from Gene	Gene	Mutation	GWAS p value	Expressed in 17 DMD Muscle Biopsies	Expressed in 6 Normal Muscle Biopsies	Expression Probeset
rs34561493	4	143043397	A/G	A	0.09	0	*INPP4B*	Synonymous S673S	0.000002	Yes (17/17)	Yes (6/6)	205376_at
rs4275414	1	182854200	A/G	A	0.27	0	*DHX9*	Intronic	0.000006	Yes (17/17)	Yes (6/6)	212107_s_at
rs72799568	16	84902483	T/A	A	0.06	0	*CRISPLD2*	Missense M294L	0.000011	Yes (17/17)	Yes (6/6)	221541_at
rs4810485	20	44747947	A/C	A	0.28	0	*CD40*	Intronic	0.000035	Yes (17/17)	Yes (6/6)	35150_at
rs6074022	20	44740196	A/G	G	0.28	6710 (5′)	*CD40*	Promoter	0.000035	Yes (17/17)	Yes (6/6)	35150_at
rs35652107	11	46339011	A/G	A	0.07	0	*CREB3L1*	Missense A411T	0.000065	Yes (13/17)	No (0/6)	213059_at
rs2014355	12	121175524	A/G	G	0.22	0	*ACADS*	Intronic	0.000069	Yes (17/17)	Yes (6/6)	202366_at
rs2281859	10	105271758	A/G	G	0.36	0	*NEURL1*	Intron - nc transcript	0.000077	Yes (17/17)	Yes (6/6)	204889_s_at
rs9333269	10	15649698	A/C	C	0.08	0	*ITGA8*	Missense Q581P	0.000096	Yes (17/17)	Yes (5/6)	235666_at

Abbreviations are as follows: SNP, single nucleotide polymorphism; GWAS, genome-wide association study; CINRG-DNHS, Cooperative International Neuromuscular Research Group Duchenne Natural History Study; Chr: chromosome; BP, base pair position (GRCh37/hg19); MAF, observed minor allele frequency; DMD, Duchenne muscular dystrophy; and nc, non-coding.

We included these 10 kb juxtagenic regions because they often contain functionally relevant regulatory elements. In fact, it has been shown by stratified false-discovery-rate analyses that SNPs situated within such distances from genes are enriched in true GWAS hits as much as coding variants, and more than intronic variants.[25] We thus selected 438 SNPs, providing a "suggestive" Bonferroni-corrected threshold of $p = 0.05/438 = 1.1 \times 10^{-4}$.

The strongest prioritized association signal corresponded to two neighboring SNPs (rs6074022 and rs4810485) in perfect LD, situated 6,710 bp upstream and in the first intron of *CD40* (MIM: 109535), respectively, on chromosome 20q. Genotypes were in Hardy-Weinberg equilibrium (HWE), and the MAF of 28% was close to expected for European ancestry (24% in the 1000 Genomes [1000G] CEU [Utah residents with ancestry from northern and western Europe from the CEPH collection] population). Median age at LoA in carriers of at least one copy of the minor allele was 2.8 years earlier (Table 2, Figure 2A), and there was a per-copy hazard ratio (HR) of 2.10 (95% confidence interval [CI] 1.45–3.04; $p = 3.5 \times 10^{-5}$). This was the only prioritized locus (NF-κB pathway) showing an association p value below the "suggestive" threshold. *CD40*, also known as *TNFRSF5* (tumor necrosis factor receptor superfamily member 5), encodes a co-stimulatory protein that is involved in T helper cell polarization and is found on the surface of antigen-presenting cells. The SNPs rs6074022 and rs4810485 are part of a non-coding haplotype spanning the 5′ region of the gene. Other prioritized SNPs with top p values are shown in Table S2 and include SNPs in *XPO1* (MIM: 602559), *PARK2* (MIM: 602544), *RIPK2* (MIM: 603455), *TLR4* (MIM: 603030), *ESR1* (MIM: 133430), *GDF5* (MIM: 601146), and *FASLG* (MIM: 134638). Association signals in the genes previously described as DMD modifiers (*SPP1* and *LTBP4*) were not picked up by the GWAS for a couple of reasons: the functional *SPP1* rs28357094 SNP has no markers in strong linkage disequilibrium on the Exome Chip; and the LTBP4 VTTT/IAAM haplotype is tagged in the Exome Chip by the rs2303729 (V194I) SNP, which because of different linkage disequilibrium in the CINRG cohort shows weaker association with LoA than rs10880 (T1140M).[7] Both of these associations had been previously confirmed in the same cohort.[7]

In order to check whether *CD40* is expressed in healthy and/or dystrophic muscle, we analyzed data from a public gene-expression dataset, the Public Expression Profiling Resource (PEPR; see Web Resources), made available from our laboratory. mRNA profiling data were generated from HG-U133 Plus 2.0 microarrays as previously described.[20] Present and absent calls were generated on the dataset (here 17 DMD and six control muscle biopsies) along with the transcript expression value via the MAS5 normalization algorithm in the Expression Console software from Affymetrix. A detection call (present, marginal, or absent) is assigned to a transcript, the reliability of which is assessed on the basis of the significant difference between perfect match (PM) and mismatch (MM) values of each

Table 2. Parameters for Kaplan-Meier and Cox Proportional-Hazards Analyses of Age at Loss of Ambulation by *CD40* rs1883832 Genotype in the CINRG Exome Chip and Validation Cohorts

Kaplan-Meier Analysis Parameters						Cox Proportional-Hazards Parameters			
	Genotype						Covariate		
Parameter	CC	CT	TT	CT and TT (dominant)	Total	Parameter	Additive Genotype	Dominant Genotype	GC Treatment
CINRG Exome Chip Cohort									
n	56	44	9	53	109	HR	2.10	2.64	0.16
Median age at LoA (years)	14.0	11.3	11.0	11.2	13.0	95% CI	1.45–3.04	1.60–4.35	0.09–0.29
95% CI	13.0–15.2	10.0–13.2	9.0–NA[b]	10.4–13.0	12.0–14.0	p value	*0.000035*	*0.0001*	*<0.0001*
CINRG Validation Cohort									
n	42	28	6	34	76	HR	1.21	1.02	0.29
Median age at LoA (years)	12.0	11.0	12.0	11.2	12.0	95% CI	0.69–2.11	0.67–1.55	0.15–0.55
95% CI	11.6–13.8	10.0–13.0	11.1–NA[b]	10.5–13.0	11.2–12.5	p value	n.s.	n.s.	*0.0002*
Bio-NMD Cohort									
n	118	98	30	128	246	HR	1.22	1.36	0.31
Median age at LoA (years)	10.6	10.0	11.0	10.0	10.5	95% CI	0.98–1.51	1.00–1.84	0.21–0.44
95% CI	10.0–11.0	9.6–10.9	10.0–12.5	10.0–11.0	10.0–11.0	p value	0.08	*0.0496*	*<0.0001*
Padova Cohort									
n	47	40	8	48	95	HR	1.20	1.19	0.41
Median age at LoA (years)	11.0	10.8	10.2	10.7	11.0	95% CI	0.81–1.79	0.75–1.90	0.25–0.67
95% CI	10.0–13.0	10.2–11.9	10.0–NA[b]	10.2–11.9	10.3–12.0	p value	n.s.	n.s.	*0.0004*
UDP Cohort									
n	139	91	13	104	243	HR	1.18	1.32	0.68
Median age at LoA (years)	10.5	9.5	11.5	10.0	10.0	95% CI	0.96–1.45	1.01–1.73	0.52–0.89
95% CI	10.0–11.0	9.0–10.0	9.5–NA[b]	9.0–10.0	10.0–10.5	p value	0.13	0.038	*<0.0001*
Overall Validation Cohort[a]									
n	346	257	57	314	660	HR	1.16	1.31	0.483
Median age at LoA (years)	11.0	10.0	11.1	10.0	10.6	95% CI	1.02–1.32	1.10–1.56	0.40–0.58
95% CI	10.5–11.0	10.0–10.5	10.2–12.0	10.0–10.5	10.2–11.0	p value	*0.02*	*0.002*	*0.005*

Abbreviations are as follows: CINRG, Cooperative International Neuromuscular Research Group; GC, glucocorticoid corticosteroids; LoA, loss of ambulation; CI, confidence interval; n.s., not significant; and HR, hazard ratio. Statistically signifcant *p* values are represented in *italics*.
[a]Sum of CINRG validation, Bio-NMD, Padova, and UDP cohorts.
[b]NA indicates that the upper limit of the confidence interval could not be estimated because the number of data points was small.

probeset. "Present call" analysis was positive on 17/17 DMD muscle biopsy samples and 6/6 healthy muscle samples (Affymetrix U133A probeset 35150_at), showing that *CD40* is expressed in both healthy and DMD muscles.

Thus, the *CD40* locus was selected for validation in independent DMD cohorts. Targeted genotyping (TaqMan) of rs1883832 (C>T, minor allele T) was used for validation. This SNP is situated in the *CD40* 5′ UTR between rs4810485 and rs6074022 and is in perfect LD with both (confirmed by TaqMan genotyping in the Exome Chip cohort).

The first validation step was performed in 108 CINRG-DNHS participants who had not been genotyped with the Exome Chip; the same statistical test as in the initial GWAS was used. Carriers of the T allele showed 1.5-year-earlier LoA (p = 0.07 for additive and 0.02 for dominant genotype effect; Figure S3). Although these data pointed toward independent validation of rs1883832 as a modifier, we were concerned about population stratification within this multi-ethnic validation cohort because some non-European ancestries, e.g., East Asian, showed both higher rs1883832 MAF (1000G) and earlier LoA in the

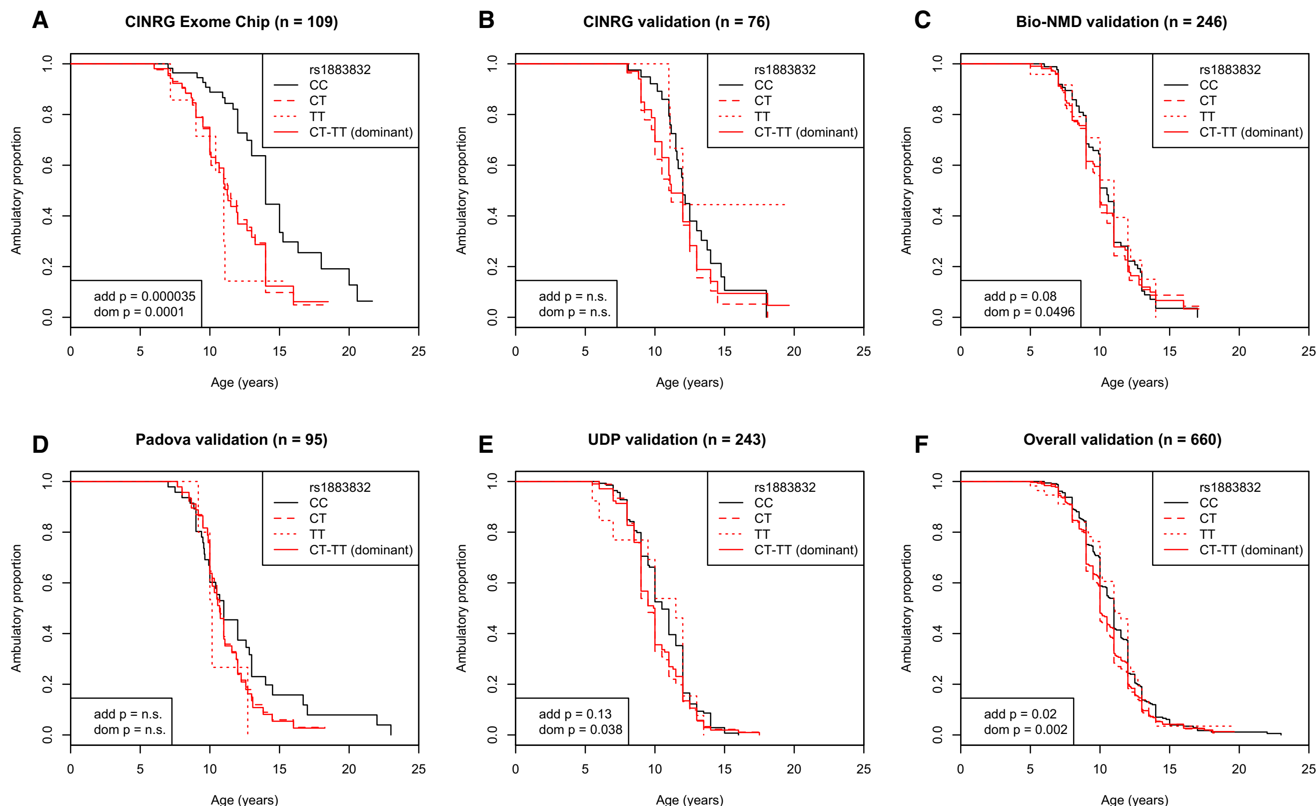

Figure 2. Kaplan-Meier Plots of Age at Loss of Ambulation by *CD40* rs1883832 Genotype
Kaplan-Meier curves are shown (additive and dominant genotype models) in the CINRG Exome Chip cohort and validation cohorts. Abbreviations are as follows: add, additive inheritance model; dom, dominant inheritance model. The overall validation cohort is the sum of CINRG validation, Bio-NMD, Padova, and UDP cohorts.

CINRG-DNHS.[7] Thus, we limited validation to 76/108 participants of self-identified non-Hispanic European race or ethnicity. Although not as effective as MDS analysis with genome-wide markers, selection by self-identified race and ethnicity is still expected to reduce population-stratification bias. In these participants, carriers of the T allele (hetero- or homozygotes) showed 0.8-year earlier LoA (non-significant p value; Figure 2B).

Subsequently, we expanded validation studies to three more independent DMD cohorts: the BIO-NMD cohort[9] ($n = 246$, European), the Padova DMD cohort[5] ($n = 95$, Italian), and the United Dystrophinopathy Project (UDP) cohort[8] ($n = 243$, mostly European American). All of these cohorts almost completely overlap with previous studies of *SPP1* and *LTBP4* associations.[5,7–9] We adopted the same statistical test as in the GWAS, except for minor differences in the definition of the binary GC treatment covariate: in the Bio-NMD and Padova cohorts, participants with any treatment duration before LoA were classified as “treated” (detailed treatment duration or dates were not available); in the UDP cohort, participants with at least 6 months of GC treatment before LoA were classified as “treated.” Both additive and dominant inheritance models were tested in validation, and statistical significance was set at $p < 0.05$. All individuals participating in each study, or their legal guardians, consented to the analysis of genetic variants for research purposes, and procedures followed were in accordance with the ethical standards of the responsible committees on human experimentation at each participating institution, as reported.[5,7–9]

In the Bio-NMD cohort, T allele carriers showed 0.6-year-earlier median LoA (Figure 2C; $p = 0.08$ [additive] and 0.0496 [dominant]). In the Padova cohort, T allele carriers showed 0.3-year-earlier median LoA (Figure 2D; non-significant p value). In the UDP cohort, T allele carriers showed 0.5-year-earlier median LoA (Figure 2E; $p = 0.13$ [additive] and 0.04 [dominant]).

The pooled validation cohorts comprised 660 participants, 50% of whom were treated with GCs. The rs1883832 SNP was in HWE; the MAF was 28% (close to expected for European ancestry). The minor T allele was associated overall with a 1-year-earlier median LoA (Figure 2F; $p = 0.02$ [additive] and 0.002 [dominant]). We added a categorical covariate for the center (CINRG, Bio-NMD Ferrara, Bio-NMD Leiden, Bio-NMD London, Bio-NMD Montpellier, Bio-NMD Newcastle, Padova, or UDP) to the Cox proportional-hazards model in this pooled validation analysis to account for cohort effects such as differences in standards of care. Survival-analysis parameters for all cohorts are summarized in Table 2.

Taken together, these findings represent a strong independent validation of the *CD40* modifier effect, suggested

by the hypothesis-prioritized GWAS. Because of a "winner's curse" effect, effect size was substantially smaller in the validation than in the GWAS cohorts (1 versus 2.8 years). Although the GWAS was run with an additive inheritance model, which is a compromise between the extreme hypotheses of completely dominant or recessive modifier SNPs, median LoA data in studied populations overall suggest a dominant model for a damaging effect of the T allele on the ambulation phenotype in DMD.

The T allele at rs1883832, adjacent to where translation starts in the *CD40* 5′ UTR (GenBank: NM001250.5 c.−1C>T), disrupts a translationally relevant Kozak sequence,[26] whereas the upstream SNP rs6074022, in perfect LD, seems to reduce *CD40* transcriptional activity evaluated in whole-blood mRNA.[27] Furthermore, the minor haplotype at this locus has been associated to increased alternative splicing of a Δ-exon-6-secreted isoform, which might act as a decoy receptor.[28] In the cited studies, the minor allele at the rs6074022-rs1883832 haplotype (here observed in association with earlier LoA in DMD) seems to downregulate CD40 signaling by both transcriptional and post-transcriptional mechanisms. The list of published genetic associations at this locus is long (see SNPaedia link in the Web Resources), including GWA and candidate-gene studies of inflammatory diseases such as Graves disease,[26] multiple sclerosis,[27] and Kawasaki disease,[28] but also of other diseases such as osteoporosis, atherosclerosis, and lymphoma; the minor allele is at times a risk factor and at times protective. There is a well-established role of T cells in pathogenesis[29–34] and response to GCs[35] in DMD, both muscle fibers and immune cells being able to present antigens to T cells.[21] CD40 is upregulated in inflammatory muscle diseases, influencing chemokine production,[36] a mechanism that might also regulate secondary inflammation in muscular dystrophies.

In order to begin to analyze the functional role of rs1883832 in dystrophic muscle, we performed rtPCR quantification of *CD40* mRNA in 16 DMD muscle biopsies from individuals with known rs1883832 genotypes in the Padova cohort (n = 4 CC, 8 CT, and 4 TT). The T allele, in a dominant model, was associated with significantly higher levels of *CD40* transcript ($p = 0.005$; Figure S4A), and trended to a lower Δ-exon-6 alternatively spliced transcript (asCD40)/CD40 ratio ($p = 0.07$; Figures S4B and S4C). We performed immunoblot quantification of CD40 protein in six muscle biopsies from the same cohort (n = 3 CC and 3 TT; smaller numbers are due to more limited access to muscle tissue than to RNA). A clear CD40 protein band at the expected molecular weight of 43 kDa for the CD40 (H10) sc-13128 antibody (Santa Cruz BT, Dallas, TX, USA) could be detected in all samples. Band intensity (normalized to tubulin) tended to be lower in "TT" biopsies, although the number of samples was low for a quantitative comparison (Figures S4D and S4E). Our mRNA expression data in DMD muscle are in the opposite direction in respect to previously published findings in whole-blood mRNA.[27] The trend observed in immunoblot, on the other hand, was in the same direction as previously published data obtained from an in vitro translation model.[26] Taken together, these findings suggest that a complex effect of the rs6074022-rs1883832 haplotype occurs at both the transcriptional and translational levels and involves tissue- and disease-specific mechanisms, which warrant further, in-depth functional studies.

The main limitation of this study was the small sample size of the initial GWAS. A larger sample size would have allowed us not only to establish associations with more certainty, but also to include analysis of rare variants with groupwise tests. Selecting phenotypic extremes, as successfully attempted in a study of cystic fibrosis (MIM: 219700) modifier variants,[37] would also have made such tests possible. Furthermore, the choice of the Exome Chip, although on the one hand facilitating the discovery of associations with functional SNPs in coding regions, on the other hand did not explore potentially relevant intergenic loci, which are better studied by traditional GWAS chips. Expanding GWASs of DMD phenotypes in larger cohorts is warranted.

In conclusion, we identified *CD40* as a modifier locus of DMD through a hypothesis-prioritized GWAS of common functional variants and validated this association in independent cohorts. Validation studies required an international collaborative effort, which represents the largest DMD association study so far. Reduced CD40-mediated cell-cell signaling in carriers of the minor rs1883832 allele might precipitate failure of regeneration and fibrosis in DMD skeletal muscle. This study points to cell-mediated immunity as a therapeutic target in DMD and represents a paradigm for the investigation of common functional variants as modifiers of rare monogenic diseases.

Supplemental Data

Supplemental Data include four figures and two tables and are available with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.08.023.

Consortia

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Conflicts of Interest

K.M.F. has served on clinical or scientific advisory boards for Sarepta Therapeutics, PTC Therapeutics, Audentes Therapeutics, Marathon Therapeutics, and Italafarmaco and has also served as a trial site investigator for PTC Therapeutics, BioMarin, Akashi Therapeutics, and Sarepta Therapeutics. A.A.-R. reports being employed by LUMC, which has patents on exon-skipping technology, and as a co-inventor on some of these patents, stands to gain from a share of potential royalties; furthermore, she reports being SAB member for Philae Pharmaceutical and ProQR, ad hoc consultant for GLC consulting, Deerfield, Global Guidepoint, BioClinica, PTC Therapeutics, BioMarin, Summit Plc, Bristol-Meier-Squibb, and a speaker at symposia organized by PTC Therapeutics and BioMarin (remuneration for consulting, SAB, and speaker activities go to LUMC). F.M. has received consulting fees from PTC Therapeutics, Sarepta Therapeutics, BioMarin, Roche, Biogen, Italfarmaco, Avexis, Pfizer, Trivorsan, and Catabasis and is supported by the National Institute of Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children's NHS Foundation Trust and University College London. H.G.-D. is a founder and shareholder of TRiNDS LLC, and a consulting statistician for AGADA BioSciences. E.P.H. is an employee and shareholder of ReveraGen BioPharma and is a founder and shareholder of AGADA BioSciences and TRiNDS LLC.

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Web Resources

Exome Chip design details URL (accessed April 7th 2016), http://genome.sph.umich.edu/wiki/Exome_Chip_Design

OMIM, http://www.omim.org

PLINK, http://pngu.mgh.harvard.edu/~purcell/plink/

Public Expression Profiling Resource (accessed April 7th 2016), http://pepr.cnmcresearch.org/

SNPaedia page for rs1883832 (accessed April 7th 2016), http://snpedia.com/index.php/Rs1883832

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