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DMD Genotypes and Motor Function in Duchenne Muscular Dystrophy

A Multi-institution Meta-analysis With Implications for Clinical Trials

Francesco Muntoni, MD, James Signorovitch, PhD, Gautam Sajeev, ScD, Henry Lane, BA, Madeline Jenkins, BSc, Ibrahima Dieye, BA, Susan J. Ward, PhD, Craig McDonald, MD, Nathalie Goemans, MD, Erik H. Niks, MD, PhD, Brenda Wong, MD, Laurent Servais, PhD, Volker Straub, MD, PhD, Michela Guglieri, MD, Imelda J.M. de Groot, PhD, Mary Chesshyre, MBBS, MA, Cuixia Tian, MD, Adnan Y. Manzur, MD, Eugenio Mercuri, MD, Annemieke Aartsma-Rus, PhD, and Association Française Contre Les Myopathies; on behalf of Universitaire Ziekenhuizen Leuven Group, PRO-DMD-01, The UK NorthStar Clinical Network, CCHMC, and The DMD Italian Group

Correspondence
Dr. Signorovitch
james.signorovitch@analysisgroup.com

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Abstract

Background and Objectives

Clinical trials of genotype-targeted treatments in Duchenne muscular dystrophy (DMD) traditionally compare treated patients with untreated patients with the same *DMD* genotype class. This avoids confounding of drug efficacy by genotype effects but also shrinks the pool of eligible controls, increasing challenges for trial enrollment in this already rare disease. To evaluate the suitability of genotypically unmatched controls in DMD, we quantified effects of genotype class on 1-year changes in motor function endpoints used in clinical trials.

Methods

More than 1,600 patient-years of follow-up (>700 patients) were studied from 6 real-world/natural history data sources (UZ Leuven, PRO-DMD-01 shared by CureDuchenne, iMDEX, North Star UK, Cincinnati Children's Hospital Medical Center, and the DMD Italian Group), with genotypes classified as amenable to skipping exons 44, 45, 51, or 53, or other skippable, nonsense, and other mutations. Associations between genotype class and 1-year changes in North Star Ambulatory Assessment total score (ΔNSAA) and in 10-m walk/run velocity (Δ10MWR) were studied in each data source with and without adjustment for baseline prognostic factors.

Results

The studied genotype classes accounted for approximately 2% of variation in ΔNSAA outcomes after 12 months, whereas other prognostic factors explained >30% of variation in large data sources. Based on a meta-analysis across all data sources, pooled effect estimates for the studied skip-amenable mutation classes were all small in magnitude (<2 units in ΔNSAA total score in 1-year follow up), smaller than clinically important differences in NSAA, and were precisely estimated with standard errors <1 unit after adjusting for nongenotypic prognostic factors.

From the Dubowitz Neuromuscular Centre (F.M., M.C., A.Y.M.), NIHR Great Ormond Street Hospital Biomedical Research Centre, Great Ormond Street Institute of Child Health, University College London, and Great Ormond Street Hospital Trust, United Kingdom; Analysis Group, Inc. (J.S., G.S., H.L., M.J., I.D.), Boston; The Collaborative Trajectory Analysis Project (J.S., S.J.W.), Cambridge, MA; Department of Physical Medicine and Rehabilitation, and Pediatrics (C.M.), University of California, Davis, Sacramento; Child Neurology (N.G.), University Hospitals Leuven, Belgium; Department of Neurology (E.H.N.), Leiden University Medical Centre, the Netherlands; Department of Pediatrics (B.W.), University of Massachusetts Medical School, Worcester; MDUK Oxford Neuromuscular Center (L.S.), Department of Paediatrics, University of Oxford, United Kingdom and Neuromuscular Center of Liège (L.S.), Division of Paediatrics, CHU and University of Liège, Belgium; John Walton Muscular Dystrophy Research Centre (V.S., M.G.), Newcastle University and Newcastle Hospitals NHS Foundation Trust, United Kingdom; Department of Rehabilitation (I.J.M.d.G.), Donders Centre of Neuroscience, Radboud University Nijmegen Medical Center, the Netherlands; Cincinnati Children's Hospital Medical Center (C.T.), and College of Medicine (C.T.), University of Cincinnati, OH; Department of Pediatric Neurology (E.M.), Fondazione Policlinico Gemelli IRCCS, Catholic University, Rome, Italy; and Department of Human Genetics (A.A.-R.), Leiden University Medical Center, the Netherlands.

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Coinvestigators are listed at links.lww.com/WNL/C620.

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Glossary

10MWR = 10-m walk/run; **ASO** = antisense oligonucleotide; **BMD** = Becker muscular dystrophy; **CCHMC** = Cincinnati Children's Hospital Medical Center; **cTAP** = collaborative Trajectory Analysis Project; **DMD** = Duchenne muscular dystrophy; **NSAA** = North Star Ambulatory Assessment; **NSUK** = North Star UK.

Discussion

These findings suggest the viability of trial designs incorporating genotypically mixed or unmatched controls for up to 12 months in duration for motor function outcomes, which would ease recruitment challenges and reduce numbers of patients assigned to placebos. Such trial designs, including multigenotype platform trials and hybrid designs, should ensure baseline balance between treatment and control groups for the most important prognostic factors, while accounting for small remaining genotype effects quantified in this study.

Duchenne muscular dystrophy (DMD) is a progressive muscle-wasting disease caused by mutations in the *DMD* gene that result in truncated, nonfunctional dystrophin protein. As an X-linked disease, DMD predominantly affects male individuals, with a pooled worldwide prevalence of 4.78 per 100,000 (95% CI 1.9–11.8).[1,2] Causative mutations include out-of-frame deletions and duplications involving 1 or more exons and nonsense mutations and small insertion and deletion mutations within exons.[3–5] Among affected boys, ambulatory deficits typically present between the ages of 3 and 5 years.[6] Although function may initially improve due to growth and development, progression of muscle pathology due to insufficient dystrophin leads to loss of ambulation, usually in the early teenage years, along with progressive losses in upper limb, pulmonary and cardiac function, and early mortality.[7–9] There is no cure for DMD. The current standard of care, which includes long-term glucocorticoid therapy, aims to manage symptoms, slow disease progression, and delay disability.[9–11]

Multiple therapeutic technologies approved or under development for DMD target specific dystrophin mutations at the DNA and (pre)-messenger RNA levels. The first targeted therapy approved for DMD (ataluren) is based on readthrough of premature stop codons during protein translation, and the therapy was conditionally approved by the European Medicines Agency in 2014.[12] In addition, a number of frameshift mutations have been targeted for drug development, particularly those occurring in hotspot regions around exons 45–55.[13] Multiple splice-modulating antisense oligonucleotides (ASOs) have been developed as exon-skipping therapies that restore reading frames in mutated dystrophin transcripts, enabling dystrophin protein expression akin to that of Becker muscular dystrophy (BMD), a clinically milder dystrophinopathy associated with in-frame deletions, duplications, or point mutations.[14–17] Accelerated approvals of exon-skipping ASOs have been granted by the US Food and Drug Administration for patients with DMD with mutations amenable to skipping of dystrophin exons 45 (casimersen),[18] 51 (eteplirsen),[19] and 53 (golodirsen[20] and vitolarsen[15]), with more under development. Due to ASO transcript and dystrophin turnover, chronic treatment is required. Currently approved ASOs require weekly intravenous dosing. Direct gene editing, based on CRISPR/Cas9, is also being investigated preclinically for DMD as a potential mutation-targeted mechanism for restoring near full-length dystrophin expression after a single treatment, although additional work to demonstrate the feasibility and safety of this approach in humans is required.[21]

As genetically targeted therapies for DMD increasingly advance to clinical trials, recruitment of patients with specific genetic subtypes within this already rare disease becomes a bottleneck for drug development. Furthermore, assigning such patients to a placebo group is understandably a source of consternation to patients, caregivers, and clinicians—especially when a drug mechanism for which proof-of-concept has already been confirmed for 1 exon-skipping ASO is extrapolated to others or when next-generation ASOs (e.g., with improved chemistry) are targeting a genotype for which a conditionally approved therapy is already available. Alongside these challenges, though, are potentially good reasons for clinical trials to include genotypically matched control groups, as has been typical in DMD trials.[12,16,19] *DMD* genotype classes have shown clinically important associations with DMD disease progression,[22–27] including differences of up to 1 or more years of age at loss of functional milestones (e.g., for patients with deletions that would be reframed with exon 44 skipping).[7,25] Genotypically matched controls aim to ensure that genotype effects do not confound treatment effects.

The intensifying practical and ethical challenges of genotype-specific recruitment and placebo exposure in DMD clinical trials prompted us to ask the following question: can the need for genotypically matched controls in DMD be reduced without sacrificing confidence in trial findings? A number of trial design solutions are attractive—but only when genotype effects are modest in magnitude, relative to expected treatment effects, and are precisely quantified. Precision is critical because a trial design that aims to account for differences in genotypes must consider uncertainty in the effects of those genotypes on outcomes, thereby adding to the overall level of noise against which a signal for drug effect needs to be

statistically detected. Greater precision means less additional noise and smaller sample sizes.

We therefore sought to estimate, as precisely as possible, the associations between genotypes amenable to skipping of exons 44, 45, 51, and 53 and 1-year changes in 2 functional outcomes used in clinical trials. To maximize precision, this collaborative study used a large collection of clinical data sources, accessed as part of the collaborative Trajectory Analysis Project (cTAP), and pooled results across these sources in meta-analyses. Based on our findings, we discuss specific trial designs that could incorporate genotypically mixed or unmatched controls in DMD.

Methods

Data Sources

Clinical data were obtained from 6 sources: curated data were collected from the neuromuscular clinic at Universitaire Ziekenhuizen Leuven (Leuven), the PRO-DMD-01 prospective natural history study (NCT01753804) for which data were provided by CureDuchenne, a 501(3)c DMD patient foundation, the iMDEX natural history study (iMDEX) (NCT02780492) funded by the Association Française contre les Myopathies, the North Star UK (NSUK) database, curated clinical practice data from the Comprehensive Neuromuscular Center at Cincinnati Children's Hospital Medical Center (CCHMC), and natural history data from the DMD Italian Group. Included patients had DMD diagnosis confirmed by genetic testing or muscle biopsy, corticosteroid treatment, and at least minimal ambulatory motor function with North Star Ambulatory Assessment (NSAA) total score ≥12 or 10-m walk/run velocity (10MWR) ≤10 seconds. Periods represented were the years 2011–2016 for Leuven, 2012–2016 for PRO-DMD-01, 2012–2018 for iMDEX, 2005–2015 for NSUK, 2004–2016 for CCHMC, and 2008–2013 for DMD Italian Group. Clinical assessments in all data sources were conducted approximately every 6 or 12 months. Additional data source characteristics, including genotyping methods, are summarized in eTable 1 (links.lww.com/WNL/C619).

Standard Protocol Approvals, Registrations, and Patient Consents

Data sources were approved by the ethics committees from each institution (University Hospitals Leuven, each participating center in iMDEX, PRO-DMD-01, and the UK NorthStar Clinical Network, CCHMC, and Catholic University, Rome). Written informed consent/assent was obtained from each participant or caregiver as appropriate before the study procedures were conducted. For the use of North Star UK data, this project followed Caldicott Guardian regulations, and information was entered in the database after written informed consent was obtained from patients' parents. Only anonymous, de-identified data were analyzed. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki, following Caldicott Guardian approval.

Dystrophin Genotypes

Patients' dystrophin genotypes were classified into sets of mutations amenable to exon skipping, nonsense mutations, and other mutations.[28,29] These classifications were confirmed by collaborators from the respective data sources. Mutations amenable to skipping of different exons were classified according to the following hierarchy to create mutually exclusive genotype classes: (1) exon 44 skip-amenable (including those amenable to both exon 44 and exon 55 skipping); (2) exon 45 skip-amenable (including those amenable to both exon 45 and exon 43 skipping); (3) exon 51 skip-amenable (including those amenable to both exon 51 and exon 53 skipping); (4) exon 53 skip-amenable (excluding those amenable to both exon 51 and exon 53 skipping); (5) other skip-amenable not included in classes (1)–(4); (6) nonsense mutations; and (7) all other mutations (i.e., not skip-amenable and not nonsense mutations).

Functional Outcomes

Time to first occurrence of timed 10MWR >10 seconds was studied as an important disease milestone that predicts loss of ambulation within 2 years (median 1 year).[30,31] This proxy was studied because completion times for 10MWR were recorded in most large data sources included in this study, whereas loss of ambulatory function was not always available.

Changes in motor function over 1 year were also studied based on the NSAA total score (ΔNSAA) and, secondarily, 10MWR velocity (Δ10MWR). The NSAA, which consists of 17 scored activities, was developed and validated for measuring motor ability in ambulatory DMD and has served as a primary and secondary endpoint in DMD clinical trials.[32] At all contributing data sources, patients' performance on each NSAA activity was scored by trained clinical staff as either 0 (unable to perform independently), 1 (performs activity using a modified method but is able to complete independently), or 2 (able to perform independently without modification). The NSAA total score is the sum of scores across all activities and ranges from 0 to 34, with higher scores indicating better function.[33]

The timed 10MWR has been used as a secondary endpoint in DMD clinical trials and was assessed by trained staff at all centers. 10MWR velocity was calculated as 10 m divided by the completion time in seconds. In CCHMC data, 10MWR completion times and velocities were approximated from recorded 30-foot walk/run times based on the relative distances of these tests.

Statistical Methods

Genotype Associations With an Ambulatory Milestone From 2 Different Time Origins

To replicate known genotype-phenotype associations[7,22–25] in our pooled database, we first studied associations between genotypes amenable to skipping of exons 44 or 51 and an ambulatory milestone (time to 10MWR >10 seconds).

Specifically, we compared age at first recorded 10MWR >10 seconds using Kaplan-Meier curves stratified by genotype with comparisons based on a log-rank test. In line with previous studies, left censoring and postindex selection bias (i.e., the fact that patients are not followed up continuously from birth and that inclusion in the studied databases may depend on outcomes) were ignored.

Second, to assess whether genotype-phenotype associations might differ in a trial-relevant setting, we analyzed time to 10MWR >10 seconds from an index date defined as the first clinic visit with 10MWR recorded and ≤10 seconds. Associations between genotype and time to milestone were quantified using hazard ratios obtained from a Cox proportional hazards model with adjustment for data source, age, and 10MWR velocity at the index date as fixed effects.

Genotype Associations With 1-Year Changes in Motor Function (by Data Source)

To address our objective of quantifying genotype-phenotype associations in a trial-relevant setting, we studied associations between selected *DMD* genotype classes and 1-year changes in NSAA total score (ΔNSAA) and, secondarily, 1-year changes in 10MWR velocity (Δ10MWR). To make use of all available data, follow-up for patients with known dystrophin genotypes was divided into approximate 1-year intervals (eFigure 1, links.lww.com/WNL/C619). Each interval was required to have (1) a baseline visit and, 8–16 months later, a follow-up visit with NSAA (or 10MWR) recorded, (2) a baseline NSAA total score ≥12 (or baseline 10MWR ≤10 seconds), and (3) nonmissing baseline prognostic factors as specified for each contributing data source (eTable 2). In each analysis, multiple nonoverlapping intervals of follow-up were allowed from individual patients. The follow-up visit from one interval could serve as the baseline visit for the subsequent interval, but no further overlap was allowed. Changes in motor function over these 8- to 16-month intervals were linearly rescaled to estimate 1-year changes, with truncation as needed based on the range of the NSAA total score.

Within each data source, associations between *DMD* genotype class and changes in motor function were estimated using regression models, with generalized estimating equations [34] and an exchangeable correlation structure used to account for within-patient correlation across multiple follow-up intervals. Three model specifications were used: (1) an unadjusted *base model*, which was common to all data sources and contained effects only for each included genotype class; (2) an adjusted *intermediate model*, which was also common to all data sources and included genotype class effects adjusted only for age and the baseline value of the outcome (NSAA or 10MWR velocity); and (3) a *fully adjusted model*, which added, to the adjustment factors included in the intermediate model, other known prognostic factors[35] available by data source (eTable 2, links.lww.com/WNL/C619). The level of variation in ΔNSAA or Δ10MWR outcomes explained by each model (R^2) was estimated separately for each data source. A fourth model was also evaluated, based on all prognostic factors included in model (3) but with genotype class removed, to better quantify the contribution of genotype class to explained variation.

Genotype Associations With 1-Year Changes in Motor Function (Pooling Across Data Sources)

Because the primary goal of this study is to estimate effects of *DMD* genotype class on motor function as precisely as possible, estimates obtained from each individual data source were combined through random-effects meta-analysis. Heterogeneity across data sources was measured using τ, the SD of cross-data source effects.

Data Availability

All relevant data are reported within the article. Data requests may be directed to the individual institutions and clinical networks that have collected and curated patient data. These organizations (Association Française contre les Myopathies Universitaire Ziekenhuizen, DMD Italian Group, CureDuchenne, UK NorthStar Clinical Network, and Cincinnati Children's Hospital Medical Center) will consider data requests according to their own data-sharing policies and governance.

Results

Genotype Associations With an Ambulatory Milestone From 2 Different Time Origins

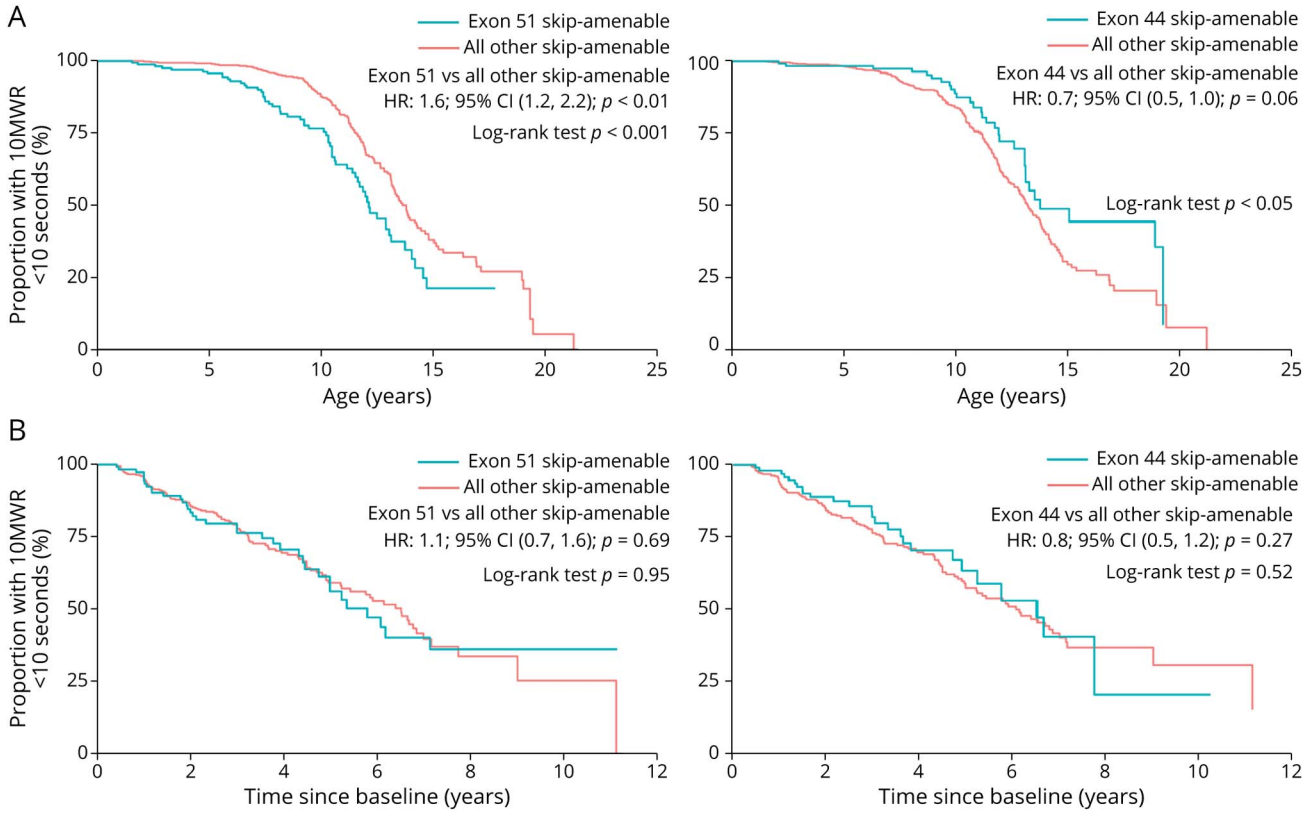
Among 962 boys, the median age at 10MWR >10 seconds was significantly younger among exon 51 skip-amenable vs all other skip-amenable mutations (12.2 vs 13.7 years, log-rank $p < 0.001$) and significantly older among exon 44 skip-amenable vs all other skip-amenable mutations (13.8 vs 13.3 years; log-rank $p < 0.05$) (Figure 1A).

By contrast, when times to 10MWR >10 seconds were measured from the first recorded 10MWR assessment ≤10 seconds, rather than from birth, genotype associations with milestone occurrence were attenuated, and not statistically significant, as indicated by overlapping Kaplan-Meier curves (Figure 1B) and hazard ratios closer to unity (eTable 3, links.lww.com/WNL/C619). Patient characteristics for these study populations are summarized in eTable 4.

Genotype Associations With 1-Year Changes in NSAA

A total of 1,668 1-year intervals of follow-up for ΔNSAA were identified from 793 unique patients. Counts by data source and by *DMD* genotype class are summarized in eTable 5 (links.lww.com/WNL/C619). Missing baseline data (i.e., missing at least 1 prognostic factor listed in eTable 2) resulted in exclusion of 25% of patients who would otherwise have been eligible. Within the study sample, all genotype classes had >120 1-year intervals from at least 59 individual patients when pooling across all data sources. Median baseline ages varied from 5 to 13 years across groups defined by both data source and genotype class (eTable 6). Median baseline NSAA total scores ranged

Figure 1 Genotype Associations With an Ambulatory Milestone (10MWR >10 Seconds) Measured as (A) Age at Milestone or as (B) Time to Milestone From First Recorded 10MWR Assessment



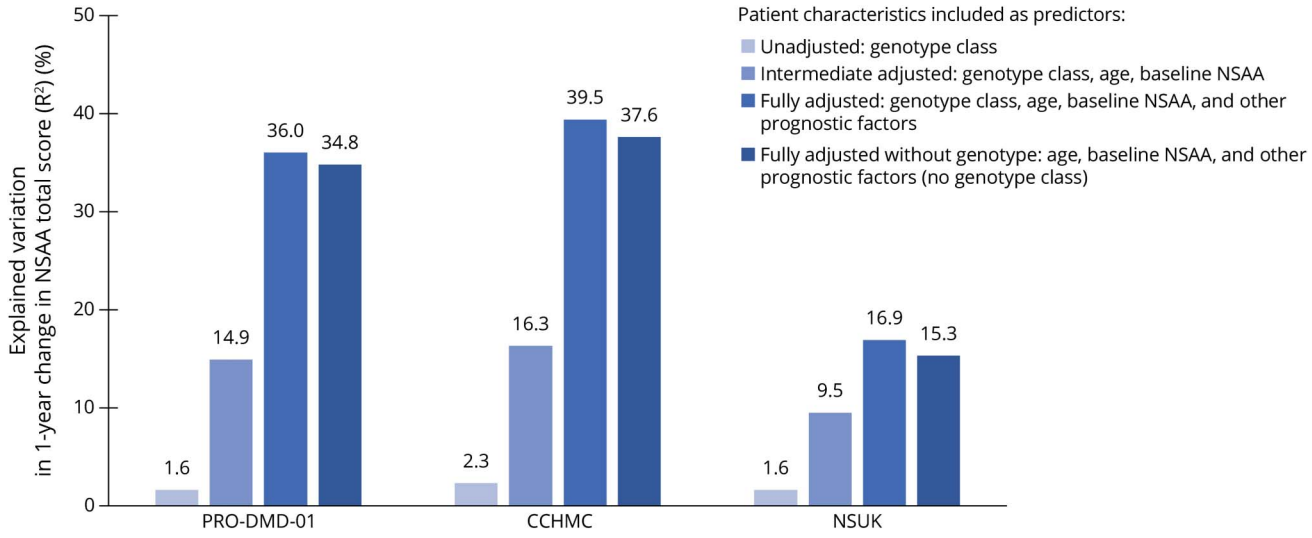
10MWR = 10-m walk/run; HR = hazard ratio.

from 17 to 31 units across these groups (eTable 7). Median 1-year changes in NSAA total score ranged from −5 to +1.8 units (eTable 8). Much of the numerical variation in these median values occurred across genotype groups with small sample sizes in specific data sources.

Studied *DMD* genotype classifications explained approximately 2% of ΔNSAA variation, both alone and when added to all other prognostic factors within the 3 largest data sources, CCHMC, PRO-DMD-01, and NSUK (Figure 2). Notably, in PRO-DMD-01 and CCHMC, the addition of multiple baseline prognostic factors in the fully adjusted model explained the most variation in ΔNSAA (R^2 of 36% and 39%, respectively), more than doubling that of the intermediate-adjusted model that accounted only for baseline age and NSAA total score in addition to genotype class (R^2 of 15% and 16%, respectively). Explained variation was lower overall in NSUK, which had fewer baseline prognostic factors available relative to CCHMC and PRO-DMD-01 (eTable 2, links.lww.com/WNL/C619). Among the 3 smaller data sources (iMDEX, Leuven, and DMD Italian Group), each of which had fewer than 5 ΔNSAA intervals in at least 2 of the genotype classes (eTable 5), explained variation due to genotype classes ranged from 6% to 21%.

Fully adjusted effects of each genotype class on ΔNSAA are depicted by data source, and after pooling through meta-analysis, in Figure 3. Pooled estimates of genotype effects were small for exon skippable mutations, consistently <2 units of ΔNSAA (Table 1). The precision of the pooled estimates was generally increased in the adjusted vs unadjusted analyses, with standard errors consistently <1 NSAA unit for all skip-amenable mutation classes. In the adjusted pooled analyses, patients with exon 51 skip-amenable mutations experienced a mean −1.3 unit (95% CI −2.3 to −0.4) difference in ΔNSAA compared with other skip-amenable patients and a mean −0.4 (−1.3 to 0.5) unit difference compared with patients with all other mutations. Patients with exon 44 skip-amenable mutations experienced mean differences of 0.3 (−0.5 to 1.1) and 0.9 (0.5 to 1.4) units ΔNSAA, relative to other skip-amenable and all others, respectively. Patients with exon 45 skip-amenable mutations experienced mean differences of 0.3 (−1.2 to 1.9) and 0.8 (−0.1 to 1.7) units ΔNSAA, relative to other skip-amenable and all others, respectively. Patients with exon 53 skip-amenable mutations experienced mean differences of −1.0 (−1.9 to 0.1) and −0.1 (−0.8 to 0.6) units ΔNSAA, relative to other skip-amenable and all others, respectively. Effects of nonsense mutations were notably more variable, with cross-trial levels of variation exceeding 3 units.

Figure 2 Percentages of Variation in 1-Year ΔNSAA Explained by Genotype Class* and Other Sets of Prognostic Factors



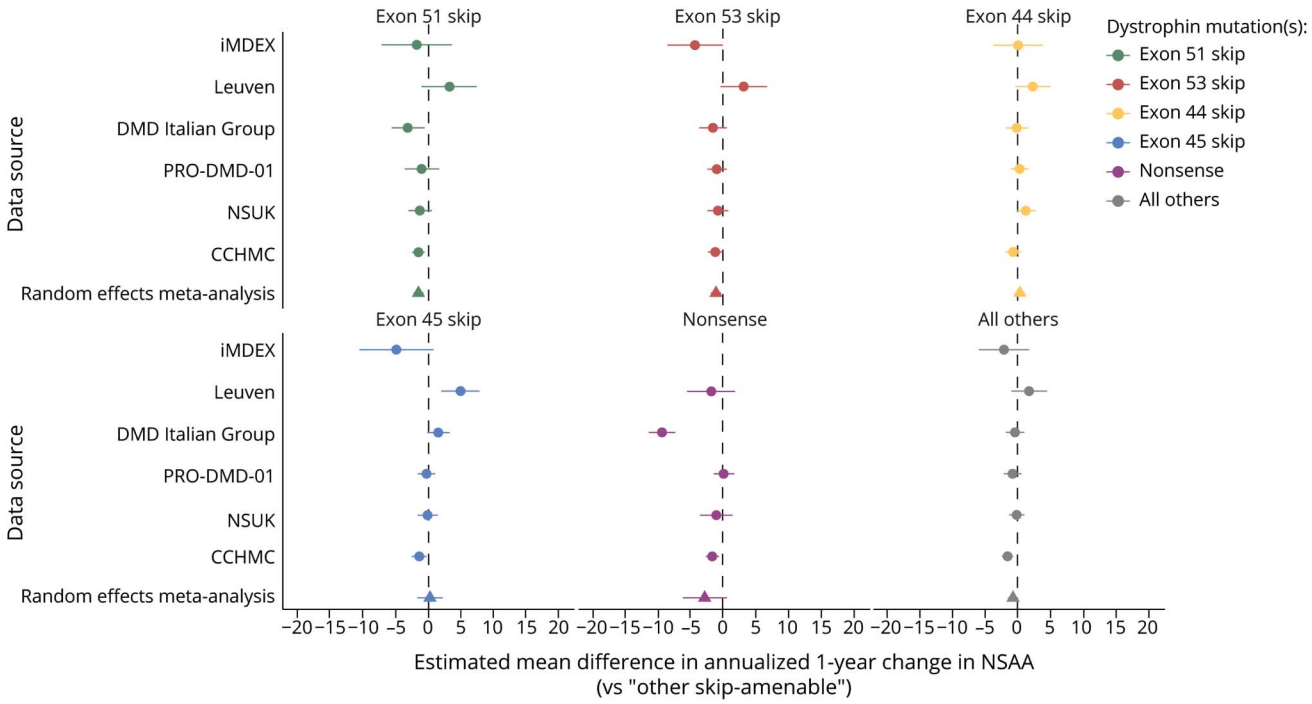
*Classified as amenable to skipping of exons 44, 45, 51, 53, other skip-amenable, nonsense and all other genotypes. CCHMC = Cincinnati Children's Hospital Medical Center; NSAA = North Star Ambulatory Assessment; NSUK = North Star UK.

Pooled genotype effects, with and without adjustment, for all genotype classes are summarized in Table 1. Variation attributable to data source effects, as indicated by τ, was also small in magnitude, at <2 units of ΔNSAA for each of the skip-amenable mutation classes studied.

Genotype Associations With 1-Year Changes in 10MWR Velocity

A total of 1,631 1-year intervals of follow-up for Δ10MWR velocity were identified from 792 unique patients. Counts by data source and genotype class for this secondary outcome are

Figure 3 Meta-analysis of Adjusted Genotype Effects on 1-Year ΔNSAA vs Other Skip-Amenable Genotypes Across Data Sources



10MWR = 10-m walk/run; CCHMC = Cincinnati Children's Hospital Medical Center; DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment; NSUK = North Star UK.

summarized in eTable 9 (links.lww.com/WNL/C619). Pooled effects from meta-analysis were consistently small, less than 0.1 m/s in magnitude (eFigure 2, eTable 10). Precision and cross-data source variation were also small, with standard errors consistently <0.1 m/s and cross-data source variation <0.2 m/s. Additional details for each meta-analysis, including data-source specific effect estimates and meta-analysis weights, are included in eTables 11 and 12.

Discussion

This study was motivated by the growing practical and ethical challenges of enrolling genotypically matched placebo arms in DMD clinical trials. Our principal finding, that often-targeted *DMD* genotype classes have small and precisely estimated effects on 1-year motor function outcomes, provides a necessary foundation for incorporation of genotypically mixed or unmatched controls into trials of such duration. It is our opinion that trial designs incorporating genotypically mixed or unmatched controls will accelerate the evaluation of genetically targeted therapies in DMD while reducing the number of treatment-amenable patients who need to receive placebo.

We first replicated known genotype-phenotype associations[7,25] in our combined data sources. Median ages at an ambulatory progression milestone were older for patients with exon 44 skip-amenable mutations and younger for patients with exon 51 skip-amenable mutations. These well-known associations may be partly due to endogenous exon skipping among patients with specific deletions.[36]

We next shifted perspective from looking at genotype associations with age at milestone (i.e., *time from birth*) to looking at associations with *time from baseline* to milestone. From this latter perspective, which is more aligned with clinical trials, the genotype-milestone associations were numerically attenuated. This may be partly explained by earlier presentation and diagnosis of patients with more rapidly progressing disease, as has been previously observed.[37] Indeed, average ages at first clinic visit in this study were younger for patients with mutations amenable to exon 51 skipping and older for those with mutations amenable to exon 44 skipping. This attenuation is a reminder that genotype associations with age at milestone are not directly applicable to the perspective of a clinical trial. When patients enroll in a trial, part of their genotype effect may already be reflected in baseline functional status, leaving less incremental effect of genotype during trial follow-up.

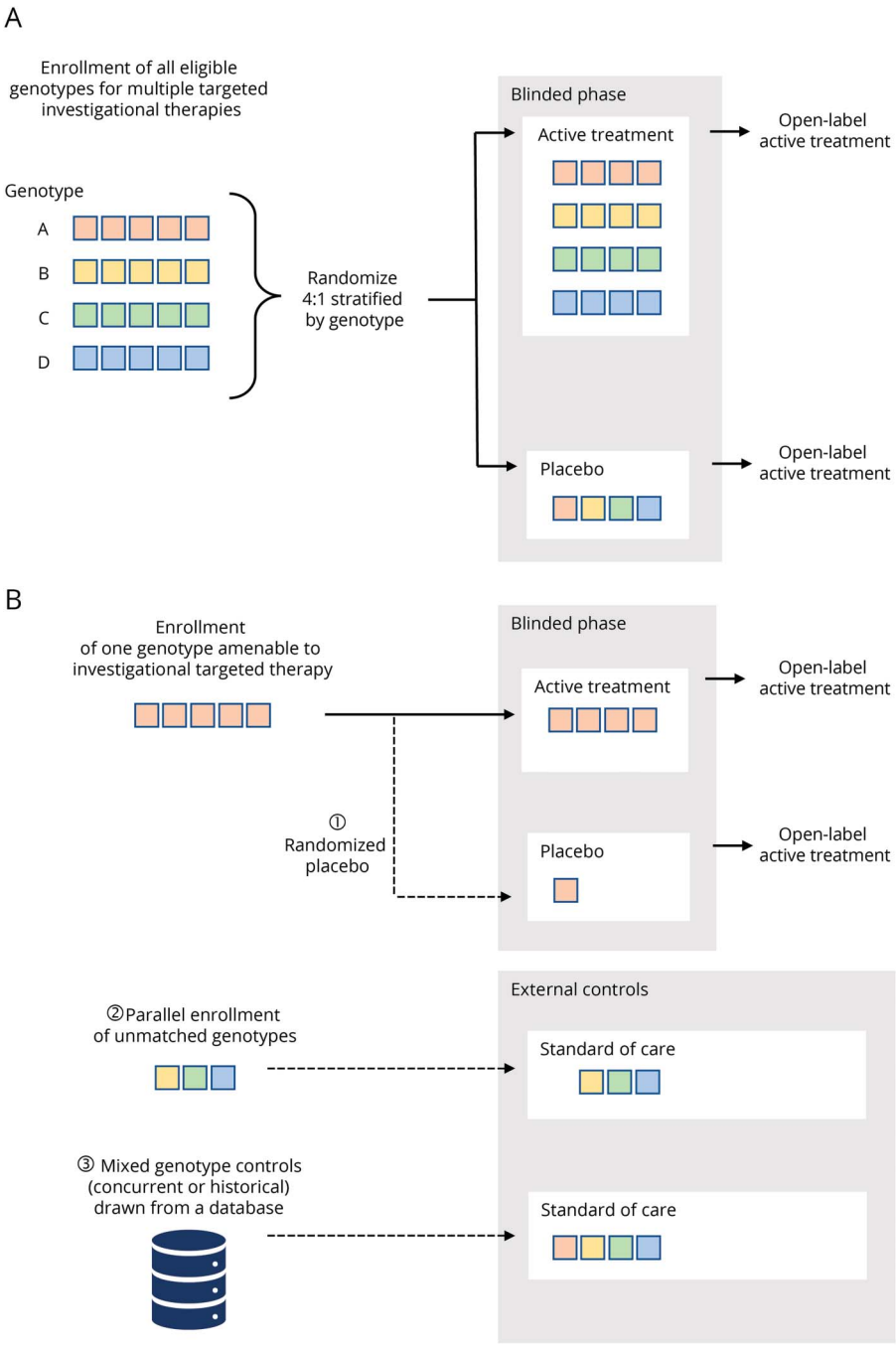
Finally, having established that shifting to a clinical trial perspective can attenuate known genotype-phenotype associations, we proceeded to the primary goal of this study: quantifying genotype effects on 1-year changes in motor function. By meta-analyzing ΔNSAA outcomes across 6 data sources representing more than 1,600 patient-years of follow-up, we established that studied genotypes have only small effects over this 1-year time

Table 1 Estimated Genotype Effects on 1-Year ΔNSAA

	Unadjusted			Adjusted		
	Estimated effect on 1-y change in NSAA (95% CI)	τ	SE	Estimated effect on 1-y change in NSAA (95% CI)	τ	SE
Effects vs other skip-amenable						
Skip 44	0.56 (−0.71 to 1.83)	1.25	0.65	0.33 (−0.47 to 1.12)	0.51	0.41
Skip 45	0.94 (−0.67 to 2.54)	1.58	0.82	0.34 (−1.23 to 1.91)	1.63	0.80
Skip 51	−0.92 (−3.24 to 1.41)	2.62	1.19	−1.34 (−2.33 to −0.35)	0.59	0.51[a]
Skip 53	−0.90 (−1.91 to 0.10)	0.58	0.51	−0.95 (−1.95 to 0.05)	0.75	0.51
Nonsense	−2.43 (−7.14 to 2.29)	5.27	2.41	−2.73 (−5.95 to 0.49)	3.50	1.64
Effects vs all others						
Skip 44	0.57 (−0.11 to 1.26)	0.46	0.35	0.94 (0.46 to 1.41)	0	0.24[a]
Skip 45	1.05 (0.19 to 1.91)	0.54	0.44[a]	0.80 (−0.11 to 1.71)	0.81	0.46
Skip 51	−0.68 (−2.30 to 0.94)	1.63	0.83	−0.39 (−1.26 to 0.48)	0.55	0.44
Skip 53	−0.78 (−1.88 to 0.32)	0.92	0.56	−0.09 (−0.80 to 0.62)	0.40	0.36
Other skip-amenable	0.11 (−1.34 to 1.55)	1.52	0.74	0.68 (−0.11 to 1.46)	0.61	0.40
Nonsense	−2.78 (−7.73 to 2.17)	5.58	2.53	−2.38 (−5.42 to 0.66)	3.32	1.55

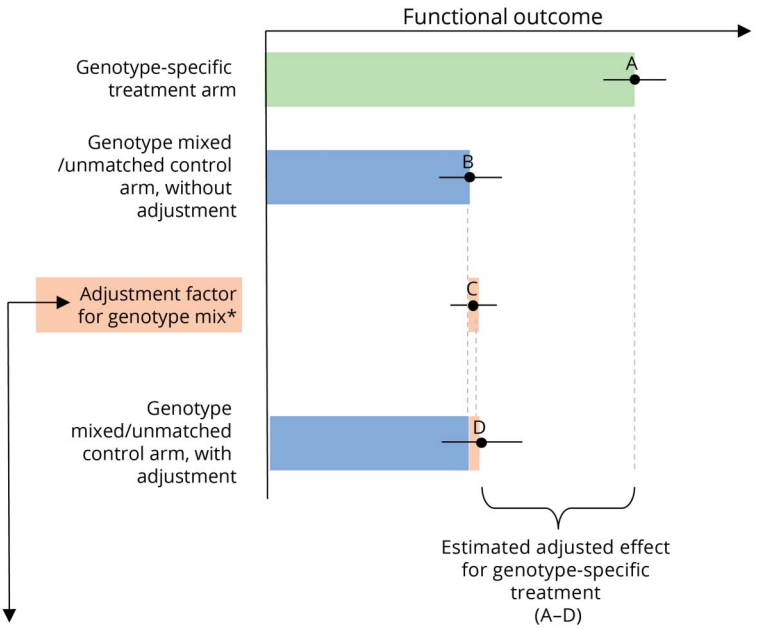
Abbreviationss: NSAA = North Star Ambulatory Assessment; SE = standard error, a measure of uncertainty in the population mean; τ = SD of the mean across data sources.
[a] $p < 0.05$.

Figure 4 Examples of Trial Designs Incorporating Genotypically Mixed or Unmatched Controls



(A) Hypothetical randomized, parallel group, blinded platform trial of multiple genotype-specific investigational therapies. In this hypothetical platform trial, patients are enrolled from 4 genotype groups (A–D) that are each amenable to 1 of 4 trialed genotype-specific investigational therapies. Patients in each genotype group are blinded to treatment assignment and randomly assigned to 1 of the 4 genotype-specific therapies or to a mixed-genotype common placebo arm in a 4:1 ratio. Comparisons of each genotype-specific therapy vs placebo are based on the shared, genotype-mixed control arm, adjusting for the genotype mix as outlined in Figure 5. This trial design could include strictly concurrent genotype-specific treatment groups (e.g., if run by a single sponsor with a multigenotype pipeline) or could admit nonconcurrent genotype-specific treatment arms (e.g., including different mechanisms and drug developers over time). The use of a shared, genotype-mixed control arm enables blinding and may reduce the overall sample size needed and the number of patients from each genotype group that are required to be assigned to placebo. (B) Hypothetical hybrid trial of a genotype-specific investigational therapy using (1) randomized genotype-matched, (2) external genotype-unmatched or (3) external mixed genotype controls. A trial of a genotype-specific investigational therapy may include different control groups: (1) concurrent, randomized, and blinded genotype-matched controls (possibly with a 1:4 or other reduced ratio of those receiving control vs active therapy), (2) external, genotype-unmatched controls, or (3) external, mixed genotype controls. Hybrid control groups can be composed of type (1) in addition to type (2) and/or type (3). Comparisons of the genotype-specific investigational therapy vs these external or hybrid control groups will require adjustment for genotype differences between groups as outlined in Figure 5 and consideration of the risk of bias due to lack of randomization and lack of blinding. In the absence of randomization, comparisons should adjust for baseline prognostic factors to mitigate the risk of bias. Bias due to unmeasured confounding cannot be ruled out in these designs, but the impacts of different magnitudes of confounding on treatment effects may be explored.[48] The risks of unblinded designs should be considered carefully, and in light of evidence showing that functional outcomes in DMD have not differed between blinded placebo arms, natural history, and real-world settings, and that adjustment for strong baseline prognostic factors can mitigate bias.[49] Inclusion of at least some randomized and blinded controls is preferred to allow direct assessment of these risks of bias.

Figure 5 Schematic for Genotype Mix Adjustment in Trial Designs Employing Mixed or Unmatched Genotype Controls



Unadjusted comparisons of functional outcomes between a genotype-specific treatment arm (A) and a mixed or unmatched genotype control arm (B) will reflect differences arising due to both treatment and the mix of genotypes across groups. Adjustment for genotype effects is therefore needed to capture differences due to treatment alone. An "adjustment factor" (C) for a specific mix of genotypes in the treatment and control arms can be calculated based on estimates of genotype effects, such as those presented in this study, and used to obtain a genotype-adjusted estimate of outcomes in the control arm (D). The estimated, adjusted effect of the genotype-specific treatment can then be calculated by comparing the genotype-specific treatment arm with the genotype-adjusted control arm (A–D).

frame, with most effects <2 units—smaller than minimal clinically important differences for NSAA[38] and smaller than the typically hypothesized treatment effect sizes over 1 year. Of importance, due to the large sample sizes studied in this collaborative research, these estimates are highly precise. Standard errors for the effects of genotype class on 1-year change in NSAA were consistently <1 NSAA unit. Effects of skippable genotypes on NSAA change in this study were directionally consistent with those reported in prior studies, with patients amenable to skipping of exon 44 or 45 progressing more slowly relative to those amenable to skipping of exon 51 or 53.[22]

We also found that genotype classes are not strong prognostic factors for 1-year functional outcomes in DMD. The studied genotypes explained only approximately 2% of variation in ΔNSAA outcomes, whereas other prognostic factors explained >30% of variation in large data sources, consistent with prior studies.[35,39] As in prior studies,[35,39,40] the strongest predictors of change in motor function (i.e., the factors that explained the most variation) were combinations of different measures of baseline motor function, that is, baseline NSAA together with some combinations of other available assessments, such as the timed rise from supine, timed rise from sitting, 4-stair climb, 10-m or 30-foot walk/run, or 6-minute walk distance. Notably, one of the prior studies has investigated prognostic factors for 1-year change in NSAA for largely the same data sources included in this study.[40]

Comparative trial designs in DMD should prioritize matching comparative groups according to strong prognostic factors, based on a data-driven understanding of prognostic strength, to improve power and avoid bias and be consistent with long-standing guidance.[41] Trial designs that risk imbalance of strong prognostic factors between comparative groups, while prioritizing balance on genotype class, should be avoided in DMD.

This study has a number of limitations. First, the genotype frequencies represented in the studied databases are not reflective of natural prevalence due to exclusion of patients enrolled in clinical trials or receiving targeted therapies available commercially or through early access programs. Sample sizes for *DMD* nonsense mutations were particularly small in some of the studied databases. Consequently, nonsense mutation patients remaining and included in this study are a small and potentially nonrepresentative subsample. Reported associations between nonsense mutations and changes in motor function, while included in this study for completeness, should be interpreted cautiously, keeping these limitations in mind.

Patients included in this study were required to have nonmissing data for multiple baseline characteristics, as listed in eTable 2 (links.lww.com/WNL/C619). This resulted in the exclusion of approximately 25% of patients who would otherwise have been eligible. While such exclusion could appear concerning, our prior research has shown that NSAA outcomes are highly consistent across these same data sources and are comparable with clinical trial placebo arms, after requiring and adjusting for data on

baseline prognostic factors.[40] Thus, we have confidence that these findings, despite exclusion of patients with missing baseline data, are representative of patients enrolled in clinical trials.

Most patients in all data sources were White. The lack of data representative of other races is an important limitation of this study.

Our study also focused on 1-year changes in function among specific genotype classes and may not generalize to longer-term follow-up or other genotype classes. Indeed, greater divergence over time in nonlinear functional trajectories across the studied exon-skippable genotypes has been well-described[22] and was also evident in the time-to-milestone analyses reported in this study for genotypes amenable to skipping of exon 44 or 51. We chose to focus on 1-year outcomes to enable as much precision as possible by pooling across all available 1-year intervals of follow-up time, recognizing that most trials in ambulatory DMD are at least 48 weeks in duration. Future studies should investigate outcomes over longer periods while pooling data across multiple sources.

Finally, not all mutations of interest for drug development could be studied adequately, even in this large pooled database study. At least 2 therapies are under development for patients with duplication of exon 2, for example. However, our study sample included only 12 patients with this rare mutation that occurs in 2% of patients with DMD. In addition, other *DMD* genotype classes and genetic modifiers at other loci[42,43] have been shown to affect functional outcomes and might also be important as prognostic factors in a clinical trial setting. Recent data suggest a potential relationship between *DMD* mutations predicted to have a differential impact on dystrophin isoform production and different patterns of motor function and age at presentation in boys with DMD,[44] and this could also play a role in genotype effects that arise during clinical trials.

Our goal of precisely quantifying trial-relevant genotype effects was accomplished by collaboratively analyzing a broad collection of data sources. Collaborating through cTAP simplified and accelerated this process and highlights the importance of data collection and data sharing for DMD drug development. While our study included data that were shared and pooled in a single location, we designed our meta-analyses approach to facilitate expansion to additional data sources without the need for sharing patient-level data across institutions.

Our estimation of small genotype effects with narrow CIs lays groundwork for several trial designs that can evaluate genotypically targeted therapies in DMD without the challenging, and in some cases prohibitive, requirement that all controls be genotypically matched to patients receiving treatment.

Platform trials, in which multiple treatments are trialed against a common control arm,[45] have been widely used in oncology settings[46] and have been considered for DMD[47] but challenged by genotype-targeted therapies. Building on the genotype effects quantified in this study, a platform trial in DMD could accommodate multiple genotype-targeted therapies by following the design illustrated in Figure 4A. An attractive feature of this design is that the genotypically mixed controls are both randomized and blinded. Hybrid trial designs that incorporate external and randomized controls have also received considerable attention in DMD. When challenged by fully matching on *DMD* genotype class, hybrid designs could use genotypically mixed external controls, as illustrated in Figure 4B. In both these designs, genotype differences could be accounted for during data analysis, leveraging the genotype effect estimates provided by this study, as illustrated in Figure 5. Alternatively, when suitably large treatment effects are expected, a genotypically mixed control group might be analyzed without genotype adjustment, provided that all stakeholders are confident that differences in genotype mix will not bias the study conclusions. Estimates of genotype effects and the precision with which they are estimated should be used to inform such confidence.

In general, sample sizes for trial designs using genotypically mixed controls will need to be larger, relative to the use of genotypically matched controls, to achieve the same power while accounting for genotype differences. However, if the increase in sample size required is modest, enrolling a larger number of genotypically mixed controls may be preferable to the challenge of recruiting a possibly smaller but fully genotypically matched control group. Additional research is underway within our collaboration to quantify power and sample size tradeoffs for designs with and without genotypically matched controls.

In applications of this research to clinical trials, it will be important to tailor adjustment for genotype effect estimates as much as possible to the specific trial setting, considering inclusion/exclusion criteria, trial duration, specific genotypes included in treatment and control groups, and adjustment for baseline prognostic factors that have larger effects on outcomes than the *DMD* genotypes themselves.

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Fowler (physical therapist; conducted functional assessments; Cincinnati Children's Hospital Medical Center, OH), Mike Kiefer (physical therapist; conducted functional assessments; Cincinnati Children's Hospital Medical Center, OH), Jean Bange (research support and data management; Cincinnati Children's Hospital Medical Center, OH), and Shengyong

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Disclosure

F. Muntoni is a member of the Rare Disease Scientific Advisory Group for Pfizer and of Dyne Therapeutics SAB and has participated in SAB meetings for PTC, Sarepta, Pfizer, Roche, Santhera, and Wave Therapeutics. UCL and Great Ormond Street Hospital have received funding from Pfizer, Italfarmaco, Wave, Santhera, Roche, NF Pharma, ReveraGen, Genethon, and Sarepta regarding clinical trials. J. Signorovitch cofounded the collaborative Trajectory Analysis Project (cTAP) and is an employee of Analysis Group, Inc., a consulting firm that received funding from the membership of cTAP to conduct this study. G. Sajeev and H. Lane are current employees, and M. Jenkins and I. Dieye are former employees of Analysis Group, Inc., a consulting firm that received funding from the membership of cTAP to conduct this study. S.J. Ward cofounded and manages the collaborative Trajectory Analysis Project and has received funding from the membership of cTAP to facilitate this study. C. McDonald has served as a consultant for PTC Therapeutics, BioMarin Pharmaceutical, Sarepta Therapeutics, Eli Lilly, Pfizer Inc., Santhera Pharmaceuticals, Cardero Therapeutics, Inc., Catabasis Pharmaceuticals, Capricor Therapeutics, Astellas Pharma (Mitobridge), and FibroGen, Inc.; serves on external advisory boards related to DMD for PTC Therapeutics, Sarepta Therapeutics, Santhera Pharmaceuticals, and Capricor Therapeutics; and reports grants from the US Department of Education/National Institute on Disability and Rehabilitation Research, National Institute on Disability, Independent Living, and Rehabilitation Research, US NIH/National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH/National Institute of Neurologic Disorders and Stroke, US Department of Defense, and Parent Project Muscular Dystrophy US. N. Goemans has received compensation for consultancy services from Eli Lilly, Italfarmaco, PTC Therapeutics, BioMarin Pharmaceutical, Pfizer, Avidity, Daiichi Sankyo, Wave, and Santhera and has served as site investigator for GlaxoSmithKline, Prosensa, BioMarin Pharmaceutical, Italfarmaco, Eli Lilly, Wave, and Sarepta. E.H. Niks is a member of the European Reference Network for Rare Neuromuscular Diseases (ERN EURO-NMD). He reported grants from Duchenne Parent Project, ZonMW, and AFM, consultancies for BioMarin and Summit, and worked as a local investigator of clinical trials of BioMarin, GSK, Lilly, Santhera, Givinostat, and Roche outside the submitted work. He reports ad hoc consultancies for WAVE, Santhera, Regenxbio, and PTC, and he worked as an investigator of clinical trials of Italfarmaco, NS Pharma, Reveragen, Roche, WAVE, and Sarepta outside the submitted work. B. Wong has participated in advisory board/committee meetings for Prosensa, Biomarin, Catabasis, and PepGen and has received compensation for consultancy services for Eli Lilly, Gilead Sciences, Pfizer, and GSK. L. Servais is member of the SAB or has performed consultancy for Sarepta, Dynacure, Santhera, Avexis, Biogen, Cytokinetics, Roche, Audentes Therapeutics, and Affinia Therapeutics. He has given lectures and has served as a consultant for Roche, Biogen, Avexis, and Cytokinetics. He is the project leader of the newborn screening in Southern Belgium funded by Avexis, Roche, and Biogen. V. Straub has participated in advisory boards for Audentes Therapeutics, Biogen, Vertex Pharmaceuticals, Italfarmaco S.p.A., Roche, Sanofi Genzyme, Sarepta Therapeutics, Summit Therapeutics, UCB, and Wave Therapeutics. He has research collaborations with Sarepta Therapeutics and Sanofi Genzyme. M. Guglieri reported serving as study chair for a ReveraGen study and the FOR DMD study (funded by the NIH). She has research collaborations with ReveraGen and Sarepta and is currently or previously acting as PI for clinical trials sponsored by Pfizer, Italfarmaco, Santhera, ReveraGen, Dynacure, Roche, PTC, and Summit. She has participated in advisory boards for Pfizer, NS Pharma, and Dyne (consultancies through Newcastle University) and performed consultancy work (speaker) for Sarepta. I.J.M. de Groot has no disclosures. M. Chesshyre has had the costs associated with attending a conference (travel, accommodation, conference fee, and food) funded by PTC Therapeutics. C. Tian has served as the

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Appendix 1 Authors

Name	Location	Contribution
Francesco Muntoni, MD	Dubowitz Neuromuscular Centre, NIHR Great Ormond Street Hospital Biomedical Research Centre, Great Ormond Street Institute of Child Health, University College London, and Great Ormond Street Hospital Trust, United Kingdom	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
James Signorovitch, PhD	Analysis Group, Inc., Boston, MA; The collaborative Trajectory Analysis Project, Cambridge, MA	Drafting/revision of the article for content, including medical writing for content; study concept or design; and analysis or interpretation of data
Gautam Sajeev, ScD	Analysis Group, Inc., Boston, MA	Drafting/revision of the article for content, including medical writing for content; study concept or design; and analysis or interpretation of data
Henry Lane, BA	Analysis Group, Inc., Boston, MA	Analysis or interpretation of data
Madeline Jenkins, BSc	Analysis Group, Inc., Boston, MA	Analysis or interpretation of data
Ibrahima Dieye, BA	Analysis Group, Inc., Boston, MA	Analysis or interpretation of data
Susan J. Ward, PhD	The Collaborative Trajectory Analysis Project, Cambridge, MA	Drafting/revision of the article for content, including medical writing for content; study concept or design; and analysis or interpretation of data
Craig McDonald, MD	Department of Physical Medicine and Rehabilitation, and Pediatrics, University of California, Davis, Sacramento, CA	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Nathalie Goemans, MD	Child Neurology, University Hospitals Leuven, Belgium	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Erik H. Niks, MD, PhD	Department of Neurology, Leiden University Medical Centre, the Netherlands	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Brenda Wong, MD	Department of Pediatrics, University of Massachusetts Medical School, Worcester, MA	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Laurent Servais, PhD	MDUK Oxford Neuromuscular Center, Department of Paediatrics, University of Oxford, United Kingdom and Neuromuscular Center of Liège, Division of Paediatrics, CHU and University of Liège, Belgium	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Volker Straub, MD, PhD	John Walton Muscular Dystrophy Research Centre, Newcastle University and Newcastle Hospitals NHS Foundation Trust, United Kingdom	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Michela Guglieri, MD	John Walton Muscular Dystrophy Research Centre, Newcastle University and Newcastle Hospitals NHS Foundation Trust, United Kingdom	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data
Imelda J.M. de Groot, PhD	Department of Rehabilitation, Donders Centre of Neuroscience, Radboud University Nijmegen Medical Center, the Netherlands	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data

Appendix 1 *(continued)*

Name	Location	Contribution
Mary Chesshyre, MBBS, MA	Dubowitz Neuromuscular Centre, NIHR Great Ormond Street Hospital Biomedical Research Centre, Great Ormond Street Institute of Child Health, University College London, and Great Ormond Street Hospital Trust, United Kingdom	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Cuixia Tian, MD	Cincinnati Children's Hospital Medical Center and College of Medicine, University of Cincinnati, OH	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Adnan Y. Manzur, MD	Dubowitz Neuromuscular Centre, NIHR Great Ormond Street Hospital Biomedical Research Centre, Great Ormond Street Institute of Child Health, University College London, & Great Ormond Street Hospital Trust, United Kingdom	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Eugenio Mercuri, MD	Department of Pediatric Neurology, Fondazione Policlinico Gemelli IRCCS, Catholic University, Rome, Italy	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Annemieke Aartsma-Rus, PhD	Department of Human Genetics, Leiden University Medical Center, the Netherlands	Drafting/revision of the article for content, including medical writing for content; study concept or design; and analysis or interpretation of data

Appendix 2 Coinvestigators

Coinvestigators are listed at links.lww.com/WNL/C620

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