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DMD Genotypes and Motor Function in Duchenne Muscular Dystrophy: A Multi-institution Meta-analysis With Implications for Clinical Trials

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ABSTRACT

Background and Objectives: Clinical trials of genotype-targeted treatments in Duchenne muscular dystrophy (DMD) traditionally compare treated patients to 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: Over 1,600 patient-years of follow-up (>700 patients) were studied from six 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, 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-meter 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 non-genotypic prognostic factors.

Discussion: These findings suggest 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 multi-genotype 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 the present study.

INTRODUCTION

Duchenne muscular dystrophy (DMD) is a progressive muscle-wasting disease caused by mutations in the *DMD* gene that result in truncated, non-functional dystrophin protein. As an X-linked disease, DMD predominantly affects males, with a pooled worldwide prevalence of 4.78 per 100,000 (95% confidence interval [CI] 1.9–11.8).[1,2] Causative mutations include out-of-frame deletions and duplications involving one or more exons as well as 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 read-through of premature stop codons during protein translation, and the therapy was conditionally approved by the European Medicines Agency (EMA) in 2014.[12] Additionally, a number of frame-shift 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 United States Food and Drug Administration (FDA) for DMD patients 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 one 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 one 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: 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 two 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 six sources: curated data collected from the neuromuscular clinic at Universitaire Ziekenhuizen Leuven (Leuven), the PRO-DMD-01 prospective natural history study [NCT01753804] for which data was provided by CureDuchenne, a 501(3)c DMD patient foundation, the iMDEX natural history study (iMDEX) [NCT02780492] was 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 (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-meter walk/run velocity (10MWR) ≤10 seconds. Time 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 in the Supplement**.

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 North Star 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 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 or exon 55 skipping); 2) exon 45 skip-amenable (including those amenable to both exon 45 or exon 43 skipping); 3) exon 51 skip-amenable (including those amenable to both exon 51 or exon 53 skipping); 4) exon 53 skip-amenable (excluding those amenable to both exon 51 or exon 53 skipping); 5) other skip-amenable not included above; 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 the majority of the 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 meters 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 two 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 post-index selection bias (i.e., the fact that patients are not followed 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 in the Supplement**). 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) non-missing baseline prognostic factors as specified for each contributing data source (**eTable 2**). In each analysis, multiple, non-overlapping 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 8–16-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 (GEEs)[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 in the Supplement**). 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)

As 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 via random-effects meta-analysis. Heterogeneity across data sources was measured using τ (tau), the standard deviation 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, North Star UK 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 two different time origins

Among N=962 boys, 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**).

In 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 in the Supplement**). 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 presented in **eTable 5 in the Supplement**. Missing baseline data (i.e., missing at least one 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 5-13 years across groups defined by both data source and

genotype class (**eTable 6**). Median baseline NSAA total scores ranged from 17-31 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 three 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 in the Supplement**). Among the three smaller data sources (iMDEX, Leuven, DMD Italian Group), each of which had fewer than five ΔNSAA intervals in at least two 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 via 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, -0.4) difference in ΔNSAA compared to other skip-amenable patients and a mean -0.4 (-1.3, 0.5) unit difference compared to patients with all other mutations. Patients with exon 44 skip-amenable mutations experienced mean differences of 0.3 (-0.5, 1.1) and 0.9 (0.5, 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, 1.9) and 0.8 (-0.1, 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, 0.1) and -0.1 (-0.8, 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. 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 10-meter walk/run 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 presented in **eTable 9 in the Supplement**. Pooled effects from meta-analysis were consistently small, less than 0.1 meters/second in magnitude (**eFigure 2**, **eTable 10**). Precision and cross-data source variation were also small, with standard errors consistently <0.1 meter/second and cross data source variation < 0.2

meters/second. 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 due in part 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 observed previously.[37] Indeed, average ages at first clinic visit in the present 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 six data sources representing over 1,600 years of follow-up, we established that studied genotypes have only small effects over this 1-year timeframe, 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. Importantly, 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 the present 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 ~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, i.e., 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-meter or 30-foot walk/run, or 6-minute walk distance. Muntoni et al. has investigated prognostic factors for 1-year change in NSAA for largely the same data sources included in the present 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, in order 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.

Limitations

The present study has a number of limitations. Firstly, 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 via 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 the present study are a small and potentially non-representative subsample. Reported associations between nonsense mutations and changes in motor function, while included here for completeness, should be interpreted cautiously, keeping these limitations in mind.

Patients included in this study were required to have non-missing data for multiple baseline characteristics as listed in **eTable 2 in the Supplement**. This resulted in 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 to 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.

The large majority of 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 non-linear 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 the present 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 time 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 two 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 DMD patients. In addition, other *DMD* genotype classes, and genetic modifiers at other loci[42,43] have been shown to impact 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.

Collaboration

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.

Applications to trial design and analysis

Our estimation of small genotype effects with narrow confidence intervals 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 the present 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 as well as randomized controls have also received significant attention in DMD. When challenged by fully matching on *DMD* genotype class, hybrid designs could employ 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 the present 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 employing genotypically mixed controls will need to be larger, relative to 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.

WNL-2022-201451_sup -http://links.lww.com/WNL/C619
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FIGURE LEGENDS AND TABLES

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

(A) Age at milestone (10MWR > 10 seconds)

(B) Time to milestone (10MWR > 10 seconds) from first recorded 10MWR assessment

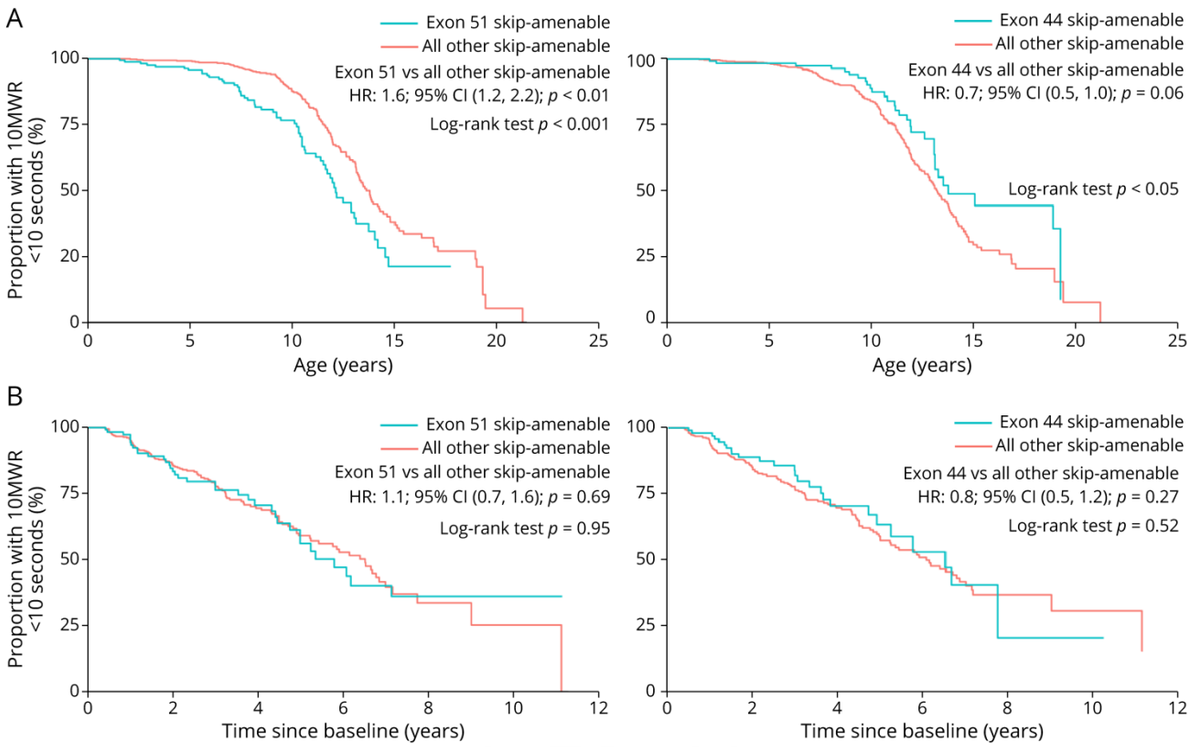


Figure 2. Percentages of variation in 1-year ΔNSAA explained by genotype class* and other sets of prognostic factors

*Classified as amenable to skipping of exon 44, 45, 51, 53, other skip-amenable, nonsense and all other genotypes.

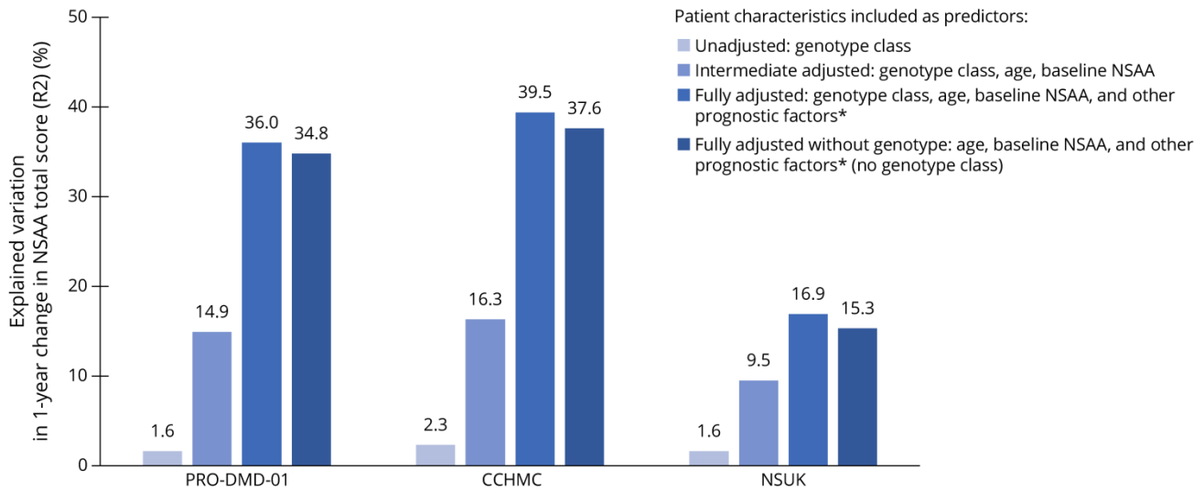


Figure 3. Meta-analysis of adjusted genotype effects on 1-year ΔNSAA vs. other skip-amenable genotypes across data sources

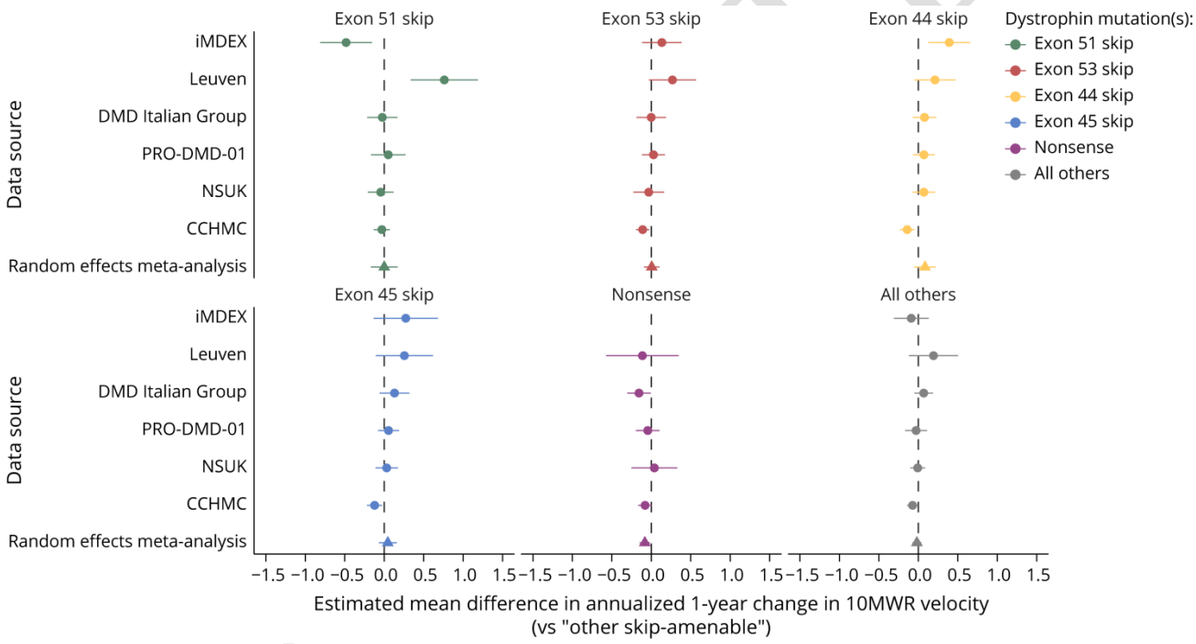


Figure 4. Examples of trial designs incorporating genotypically mixed or unmatched controls

Figure 4A: Hypothetical randomized, parallel group, blinded platform trial of multiple genotype-specific investigational therapies

In this hypothetical platform trial, patients are enrolled from four genotype groups (A, B, C, D) that are each amenable to one of four trialed genotype-specific investigational therapies. Patients in each genotype group are blinded to treatment assignment, and randomly assigned to one of the four genotype-specific therapies or to a mixed-genotype common placebo arm in a 4:1 ratio. Comparisons of each genotype-specific therapy versus 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 multi-genotype pipeline) or could admit non-concurrent 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.

Figure 4B: Hypothetical hybrid trial of a genotype-specifc investigational therapy using (1) randomized genotype-matched, (2) external genotype-unmatched or (3) external mixed genotype controls

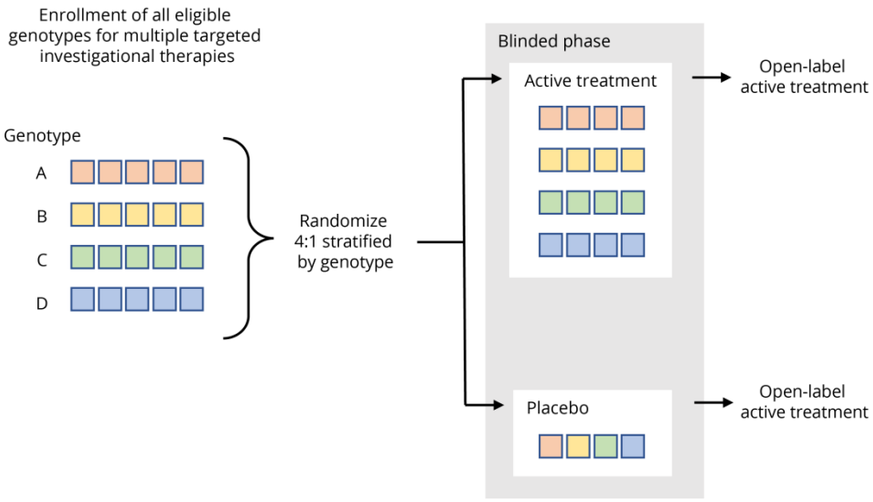
A trial of a genotype-specific investigational therapy may include different control groups: (1) concurrent, randomized and blinded genotype-matched controls (possibly with a 1:4 or other reduced ratio of those receiving control vs. active therapy), (2) external, genotype-unmatched controls, or (3) external, 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 versus these external or hybrid control groups will require adjustment for genotype differences between groups as outlined in Figure 5, as well as 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 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.

A



B

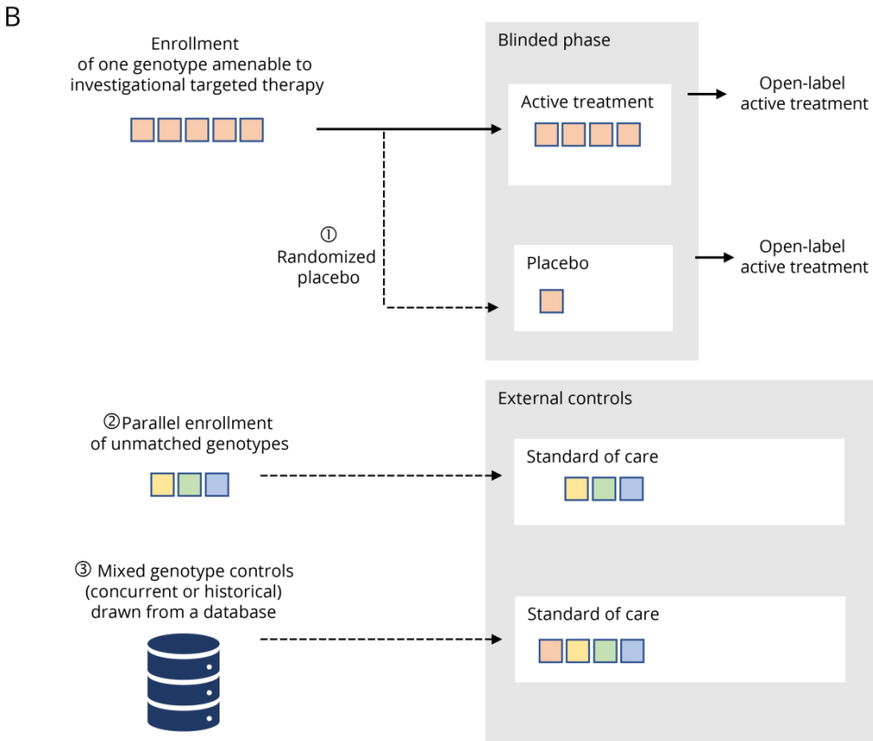


Figure 5. Schematic for genotype mix adjustment in trial designs employing mixed or unmatched genotype controls

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 to the genotype-adjusted control arm (A - D).

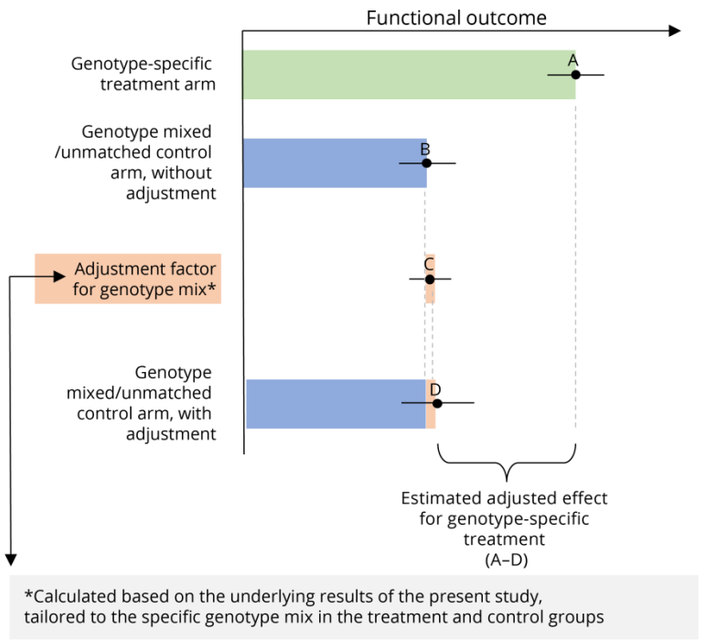


Table 1. Estimated genotype effects on 1-year ΔNSAA

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

*p-value < 0.05;
T = the standard deviation of the mean across data sources
SE = standard error, a measure of uncertainty in the population mean

DMD Genotypes and Motor Function in Duchenne Muscular Dystrophy: A Multi-institution Meta-analysis With Implications for Clinical Trials

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