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21st Century advancements in Exon Skipping based therapeutic approaches for Duchenne Muscular Dystrophy: a meta-analysis

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Abstract
Duchenne Muscular Dystrophy (DMD) is a severe X-linked genetic disorder affecting approximately 1 in 3,600 male births worldwide. It develops through several different types of mutations in the DMD gene, resulting in the insufficient production of the protein dystrophin, which is essential for muscle contraction and relaxation. A loss of dystrophin results in early loss of mobility, progressive muscle degeneration and ultimately, premature death. Symptoms of DMD initially emerge in early childhood including those of delayed motor milestones and muscle weakness. Eventually, assistive devices like wheelchairs become necessary. Patients with DMD report a much lower Quality of Life (QoL), mostly as a result of social and physical difficulties. Gene therapy, especially exon skipping therapy, has shown promise despite the lack of a cure. This strategy modifies the pre-mRNA transcripts using antisense oligonucleotides (AONs) in a the hope of restoring dystrophin production. Patients now have new hope as severe DMD cases have been transformed into the milder Becker Muscular Dystrophy phenotypes through initial clinical trials targeting exon 51. Exon skipping effectiveness, however, varies, and its applicability is limited by mutation-specificity and uneven dystrophin restoration levels. This meta-analysis was conducted in order to assess the biochemical effects of exon skipping on dystrophin levels in DMD patients. The analysis, which utilized a random-effects model, showed a considerable increase in dystrophin expression for those clinical trials that utilized exon skipping. However, the analysis also emphasized the necessity of further research to address treatment heterogeneity and improve longer-term outcomes. The results highlighted the value of long-term therapeutic interventions and personalized medicines in enhancing DMD patient quality of life and treatment efficiency.

Keywords
Duchenne Muscular Dystrophy, Exon Skipping, Genetic mutation, Biochemical restoration, Quality of Life, Antisense oligonucleotides, Dystrophin, Meta analysis, Eteplirsen, Effect size

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Introduction

Duchenne Muscular Dystrophy (DMD) is a severe X chromosome-linked genetic disorder characterized by rapid muscle degeneration, affecting approximately 1 in 3600 male births worldwide (1). This condition arises from various different types of mutations in the DMD gene, in which ~ 60-70% are large mutations, surrounding the deletion and/or duplication of one or more exons, while the rest, ~ 25-35% or so, are composed of smaller mutations, such as missense, nonsense, and frameshift ones (2). A key role of the DMD gene is to encode for dystrophin, a protein which is essential for muscle contraction and relaxation. Without sufficient dystrophin, the individual's muscle cells are damaged, leading to greater progressive weakness and even early mortality (3). The start of these symptoms typically occurs in early childhood. Initial signs include muscle weakness and delayed motor milestones, eventually leading to difficulties in walking, frequent falls, muscle cramps, and respiratory complications. The disease follows a predictable pattern with muscle weakening worsening over time, leading to the patient often needing the support of external aid such as wheelchairs, or braces to maintain their independence (1). Due to this progressively weakening disease, the Quality of Life (QoL) of DMD patients is significantly lower than average. Lue et al. found a lower QoL on the WHOQOL-BREF (World Health Organization Quality of Life Brief Version) in two key domains: i) Physical. ii) Social (4). Though this study was conducted in Taiwan, it found the loss of arm function (a common progression of DMD patients globally) to be one of the major factors contributing to this low QoL, emphasizing that the reduced quality of life is not specific to just Taiwan but rather to DMD patients overall.

Common complaints included the negative psychological impact of feelings of loss of the patient's condition (5,6). Furthermore, the disease is associated with an extremely large economic burden. Landfeldt et al. found that, on average, DMD households lose between $58,440 and $71,900 more per year compared to regular households, due to expensive treatments, greater number of work hours missed and other day-to-day sacrifices (7). Ironically, on the other hand - given that DMD caregivers reported worse mental health and a stronger economic burden, compared to parents without a DMD child - caregivers displayed significantly better physical health due to the patients dependency on a wheelchair for mobility (6). The devastating impact of DMD, not just on the 20,000 patients diagnosed each year but also on each of their families, highlights the critical need for therapeutic interventions.

Exon skipping is a promising advance due to its potential to directly address genetic mutations underlying the disease (8,9). This therapeutic strategy aims to produce a truncated but functional form of dystrophin, potentially slowing, if not halting the disease's progression (9). The first clinical trial for exon skipping in DMD began in 2006, and marked a significant advancement in DMD therapy. This trial utilized AONs designed to skip exon 51 of the dystrophin gene (10). The objective was to transform the more severe DMD phenotype into its milder counterpart, Becker Muscular Dystrophy (BMD), which is often associated with less severe symptoms and a slower

progression of muscle weakness (10-12). The trial was an important point for DMD patients, as it gave them new hope by applying new-found molecular genetic insights directly into therapeutic interventions. This initial trial set the basis for future research and development in exon skipping therapies. A notable clinical trial for exon skipping therapy was the first trial for Eteplirsen in 2013. This double-blind, placebo-controlled study analyzed eteplirsen's efficacy in increasing dystrophin-positive fibers and improving functional outcomes, as measured by the 6-minute walk test. Patients were randomized into placebo, 30 mg/kg/week, or 50 mg/kg/week groups, with placebo patients switching to active treatment after 24 weeks. Key findings showed increased dystrophin-positive fibers after 48 weeks. However, two patients with rapid disease progression were excluded from the analysis as outliers (13). This exclusion enhanced the apparent effectiveness of Eteplirsen by removing data points that would have reduced overall efficacy, and instead manufactured a clinical significance. While methodologically justifiable, this decision highlighted the variability in treatment response among DMD patients and raised questions about the actual effectiveness of Eteplirsen. Despite these concerns, Eteplirsen was approved by the FDA, although not without significant internal disagreements. Dr. Janet Woodcock, Director of CDER, supported the accelerated approval based on the totality of the evidence approach, considering the life-threatening nature of DMD and the lack of alternative treatments. On the other hand, Dr. Unger, Director of the Office of Drug Evaluation 1, opposed the approval, arguing that the data did not sufficiently demonstrate that the increase in dystrophin was "reasonably likely" to predict any clinical benefit (14). In the end, despite the lack of concrete scientific evidence, the drug was approved due to the urgent needs of DMD patients and community pressure. However, this approach was controversial, with some critics arguing that it set a dangerous precedent for lowering approval standards by valuing community pressure over scientific validation. The development of other exon-skipping therapies like Drisapersen has also faced challenges typical of clinical research for rare diseases. The rarity of DMD makes it difficult to recruit sufficiently large and representative patient samples, leading to broad inclusion criteria that increase heterogeneity. Furthermore, the need to include numerous investigation sites introduces variability in standards of care and data collection, complicating the assessment of therapeutic efficacy (15).

Over the 21st century and in recent years, exon skipping has been reformed across various clinical trials, exploring different exon skipping inducers and optimizing the use of AONs (9,16). These studies are conducted either *in vivo*, within living organisms, or *in vitro*, outside the organism using isolated cells (17). These studies have not only shed light on the potential of exon skipping to be able to result in clinically meaningful outcomes but have also shown the complexity of this treatment, requiring precise targeting and delivery mechanisms to achieve sustained dystrophin expression (18). A common metric of measuring this dystrophin restoration is by western blot bands, specific protein markers visualized on a gel, which quantify the presence and amount of dystrophin protein in a

sample. Their numbers are generally also quantified in a graph or a table (19). Other studies also display temporary effects of the treatment, requiring ongoing therapy to maintain benefits. These hurdles and challenges highlight the need for continued research to refine and enhance the exon skipping approach for broader and more effective applications in DMD therapy.

As of the writing of this manuscript, there was only one published meta-analysis from 2018 that compiled the effects of exon skipping on Duchenne Muscular Dystrophy (20). This analysis, while extremely valuable, primarily focused on the physical effects of exon skipping treatment such as the 6-minute walk test (6MWT) distance, adverse events, and other functional outcome measures but did not assess the biochemical changes which are crucial for a more comprehensive understanding and evaluation of therapeutic efficacy. Furthermore, in the six years following this publication, significant advancement in medical technology–including the development of artificial intelligence and enhancements in overall gene therapy– have emerged, highlighting the need for updated research that incorporates new trials integrating these technological innovations (21,22). This gap in the existing literature and the evolution in the field of medical science emphasize the necessity for a new meta-analysis. This research aims to focus on more biochemical changes following the treatment, concentrating on the percent of dystrophin restored due to the exon skipping based intervention. By synthesizing both recent and earlier studies, this research aims to deliver an up-to-date understanding of the potential of exon skipping treatment for DMD, by combining various findings into a unified idea of efficacy.

Methods

Search strategy

This meta-analysis was reported in accordance with the checklist set in the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA). For the retrieval of articles, a systematic literature search was conducted in two peer-reviewed online databases, ScienceDirect and EBSCOHost, and one register http://ClinicalTrials.gov, for access to gray literature (unpublished work to reduce publication bias). The following key terms were input into the two databases: (“Gene therapy” OR “genetic intervention”) AND ("DMD" OR "Duchenne Muscular Dystrophy") AND ("exon skipping" OR "exon-skipping therapy" OR "exon exclusion" OR “exon modulation.”) For the register the disease was selected as “Duchenne Muscular Dystrophy” and the intervention was selected as “Exon skipping.”

Inclusion and exclusion criteria

In this meta-analysis the sources resulting from the key words were assessed. Open access full-text linked research articles with results communicated in the English language from the year 2000 and onwards were included. Other types of articles including review articles, book chapters, conference abstracts, case reports, editorials, reports, and other meta-analysis were excluded. Once narrowed, the included studies were independently screened by two reviewers for eligibility on the basis of full text using PICOS (23). The criteria for *P*opulation were human and animal individuals diagnosed with Duchenne Muscular Dystrophy

(DMD), either pediatric and/or adult populations. The criteria for *I*ntervention were studies investigating exon therapy intervention for Duchenne Muscular Dystrophy (DMD) and studies including both *in vivo* and *in vitro*, or only *in vivo* studies. The criteria for *C*omparison were Mdx Mice model, Dup2 Mice Model, BL6 (healthy) mice *vs.* the placebo. The *O*utcome measures included studies reporting relevant clinical outcomes, including quantifiable western blot bands for dystrophin expression, and studies reporting safety and adverse effects related to specific exon skipping treatment. The *S*tudy design included randomized controlled trials. Any disagreements were resolved between the two reviewers in a discussion.

Data extraction

For each study, which passed through the two phases of selection criteria, data was extracted by two reviewers working independently in the order, 1] References (Title, Author(s), Year, and other publication details), 2] Goal, 3] Exon Therapy intervention details (type of exon skipping used, administration method, dosage), 4] Outcome Measures (Dystrophin levels restored, time points of measurements, potential adverse effects), 5] Additional relevant information, 6] Conclusion and impact on DMD.

The outcome measure extracted in this study was the percent of dystrophin levels restored on a Bl6 standard curve. Data needed to run a standardized mean difference test, including mean of treatment, standard deviation (SD) of treatment, sample size of treatment along with the same three parameters for control groups was extracted. Considering that several studies did not report a mean or SD for their control groups, a weighted average of all studies that reported mean and SD for control groups (n=3) was used as a baseline for each study and treatment with varying sample sizes based on individual study reports.

Data analysis

All analyses were performed using the metafor package in the R Statistical Software (v4.3.3; 2024-02-29 ucrt) (24,25). Standardized mean differences were calculated for primary outcome measures using a random-effects model which simply accounts for between-study and across-study variability. Further, in this meta-analysis, the "range method" was utilized to estimate missing standard deviations (SDs) for a few of the studies where these values were not reported. This approach, initially described by Mendenhall et al. within the survey sampling framework, involved dividing the difference between the maximum and minimum observed SD values by 4 to approximate the SD. This method enabled the inclusion of all relevant studies, ensuring a more comprehensive analysis despite the absence of complete data for some cases (26). The results of this SMD data were visually represented in a forest plot, a graphical representation used commonly in meta-analyses to visually display the individual results of each study or treatment along with the overall combined statistics, showing effect sizes and their confidence intervals (27). Heterogeneity, which refers to the variability among the included studies, was quantified using the I^2 statistic as well as Cochran's Q test. Meta-regression analyses, which is a tool used in meta-analysis to examine the impact of study-level characteristics on the effect size

across different studies, was utilized to try and explore the impact of total sample size on the effect sizes (28). Specifically, the logarithm of the total sample size (log_n) was used as a moderator to explore its relationship with the intervention's effectiveness. This was then converted into a plot displaying the results of the meta regression. Subgroup analyses were also conducted based on the year of study publication, categorizing studies into two groups: “Before 2020” and “After 2020.” This separation was performed to examine the more temporal trends in the effectiveness of the intervention. To further explore the causes of heterogeneity, a second subgroup analysis was conducted categorizing the time points of measurements of dystrophin levels into two groups: “Before 48 weeks" and “After 48 weeks.” A leave-one-out sensitivity analysis was also conducted to find the strength of the meta-analyses findings. This involved progressively excluding each individual study and recalculating the pooled effect size to identify any study’s disproportionate influence on the overall results. The results of this leave-one-out analysis were then plotted and converted into a visual representation. To assess reporting bias (publication bias) a funnel plot was created, with results supported by Egger's regression test, a statistical tool used to assess the symmetry of a funnel plot, particularly assessing the relationship between the effect sizes and their standard errors (29). Asymmetry in the funnel plot and a significant Egger's test result is indicative of potential publication bias.

Results

Search results and study characteristics

The initial search identified 1,164 total results, out of which only ~ 300 remained after removal based on the inclusion criteria mentioned. After initial screening for eligibility based on titles and abstracts as well as removal of duplicates, 30 (10.5%) articles remained for further evaluation of the full text on the basis of the eligibility criteria with a focus on including studies reporting quantified dystrophin levels through western blot analysis. Of these 30, 11 (36.67%) articles met all the eligibility criteria evaluated and hence were included in the study. Pertaining to the selection criteria based on relevance, only studies centered on exon skipping with quantified outcome measures were selected, while studies with brief mentions of the prior mentioned focus, along with studies investigating other outcome measures or types of gene therapies were deemed as unsuitable for the meta-analysis.

In summary there were eleven studies selected, 5 from ScienceDirect, 0 from EBSCOhost due to removal of duplicates, and 6 from http://ClinicalTrials.gov (Figure 1). Many of the studies were performed *in vivo* (n=10; 90.91%), while one study (9.1%) was performed in combination of *in vitro* and *in vivo*. All studies (100%) reported quantifiable results of western blot bands for dystrophin protein expression either through tables or bar graphs. The outcome measure, percent of dystrophin restored on a Bl6 standard curve, served as a uniform metric across studies facilitating direct comparisons.

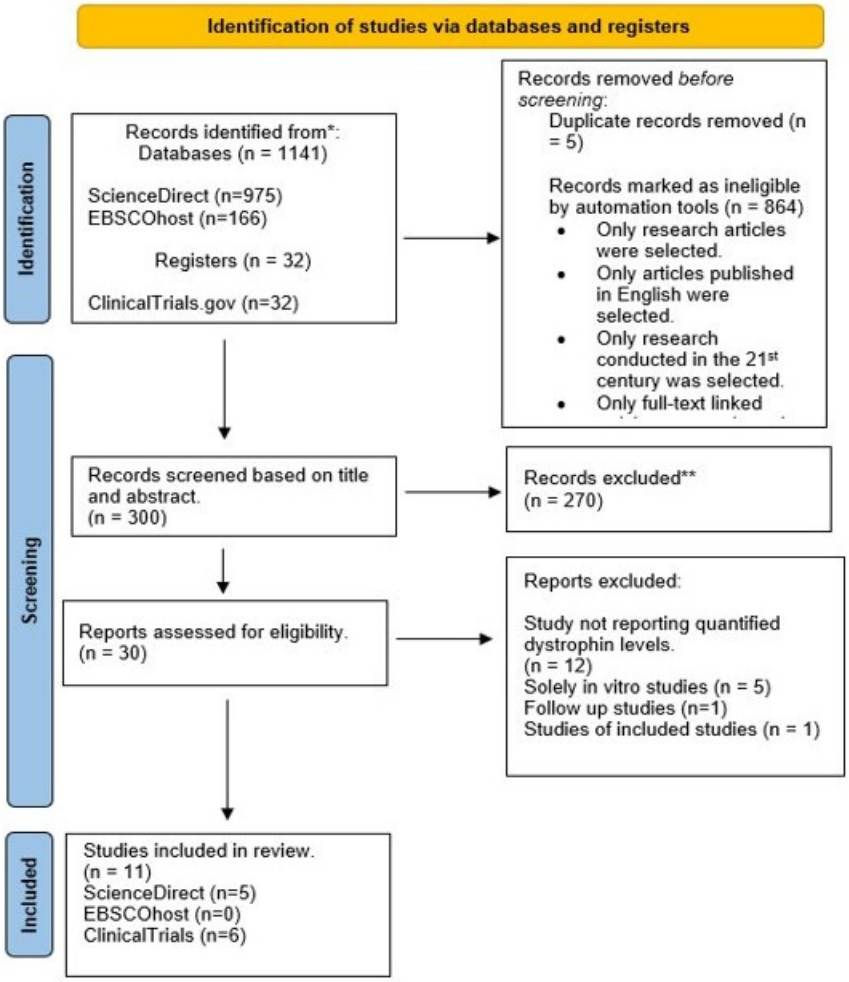

Figure 1. Flow chart representing the study selection process per PRISMA

Heterogeneity and overall effect size

Extremely high heterogeneity ($Q = 412.53$, $df = 16$, $p < 0.0001$; $I^2 = 97.2\%$) was found in the statistical analysis, demonstrating that variations in effect sizes were 97.2%; not the result of chance. This led to the application of a random-effects model, which provides a more sophisticated calculation of the total treatment impact across all participants (n=301) by taking into account both within-study, and between-study variance.

Despite the pronounced heterogeneity, the pooled random-effects model estimate of the treatment effect was substantial and statistically significant, with an effect size of 1.9237 ($SE = 0.6443$, $z = 2.9856$, $p = 0.0028$), and a 95% confidence interval ranging from 0.6609 to 3.1866, suggesting that exon-skipping

interventions led to a significant increase in dystrophin expression (Figure 2). Individual study effect sizes ranged from largely negative (Hedges' g = -2.17) to largely positive (Hedges' G= 7.04) again pointing to a high level of heterogeneity.

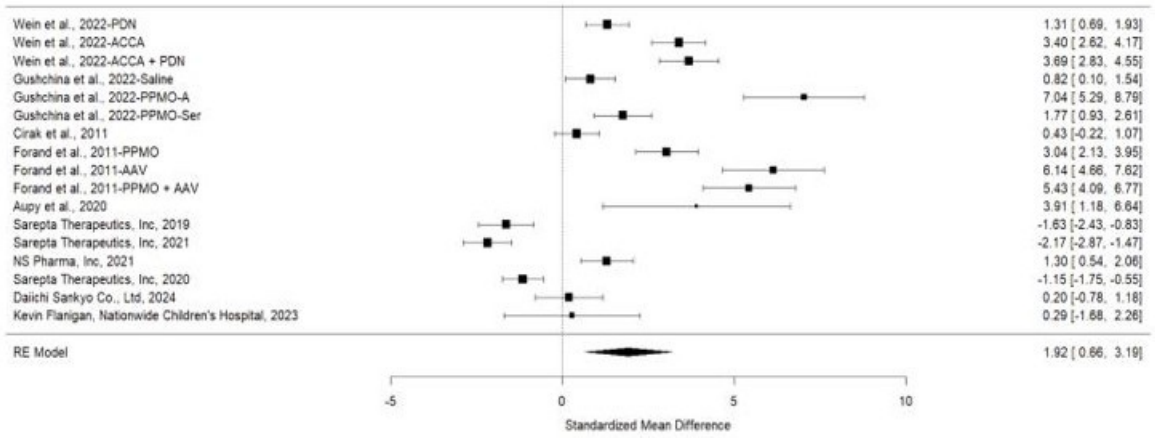

Figure 2. Graphical representation of individual treatment effect sizes with confidence intervals

Publication bias assessment

The funnel plot exhibited some asymmetry (Figure 3). and the Egger's test provided statistical evidence for funnel plot asymmetry (z = 2.3297, p = 0.0198), suggesting the presence of publication bias or other small-study effects. The estimated effect as the standard error approached zero was -0.6848, with a 95% confidence interval ranging from -3.1292 to 1.7595, indicating a trend towards smaller studies reporting more favorable treatment effects.

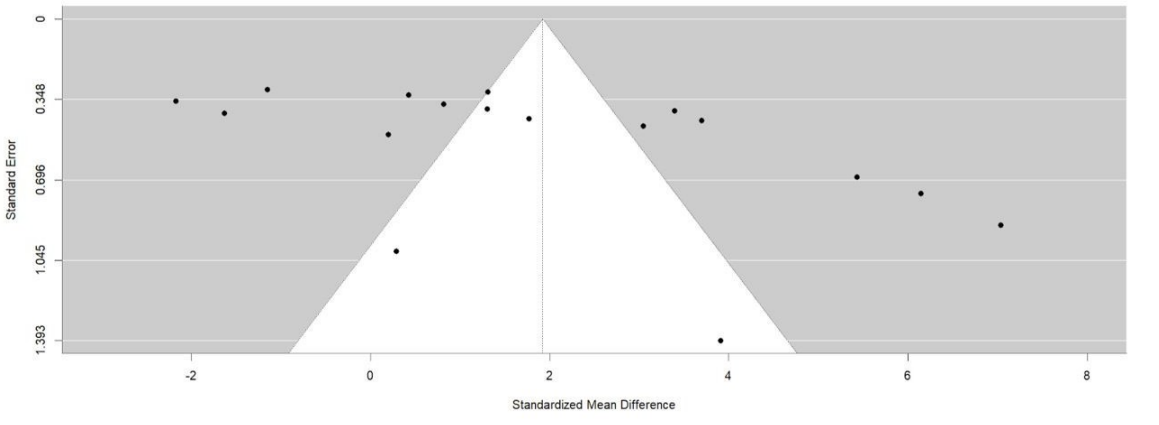

Figure 3. Semi-asymmetrical funnel plot assessing publication bias.

Sensitivity analysis

Sensitivity analyses were performed to assess the impact of individual studies on the overall effect size and heterogeneity. The leave-one-out approach, where each study was sequentially removed from the meta-analysis, indicated that no single study disproportionately influenced the meta-analysis outcomes (Figure 4). For instance, exclusion of study one resulted in a pooled effect size of 1.9677 (SE = 0.6869, z = 2.8646, p = 0.0042), and the heterogeneity measures remained high (I^2 = 97.22%, τ^2 = 7.1648). This pattern was consistent across all studies removed in the sensitivity analysis, with all p-values remaining well below the 0.05 threshold, ensuring the robustness of the meta-analytic findings. This indicated that no single study had a significant impact on the variability observed, prompting further investigation to assess the causes of this variability.

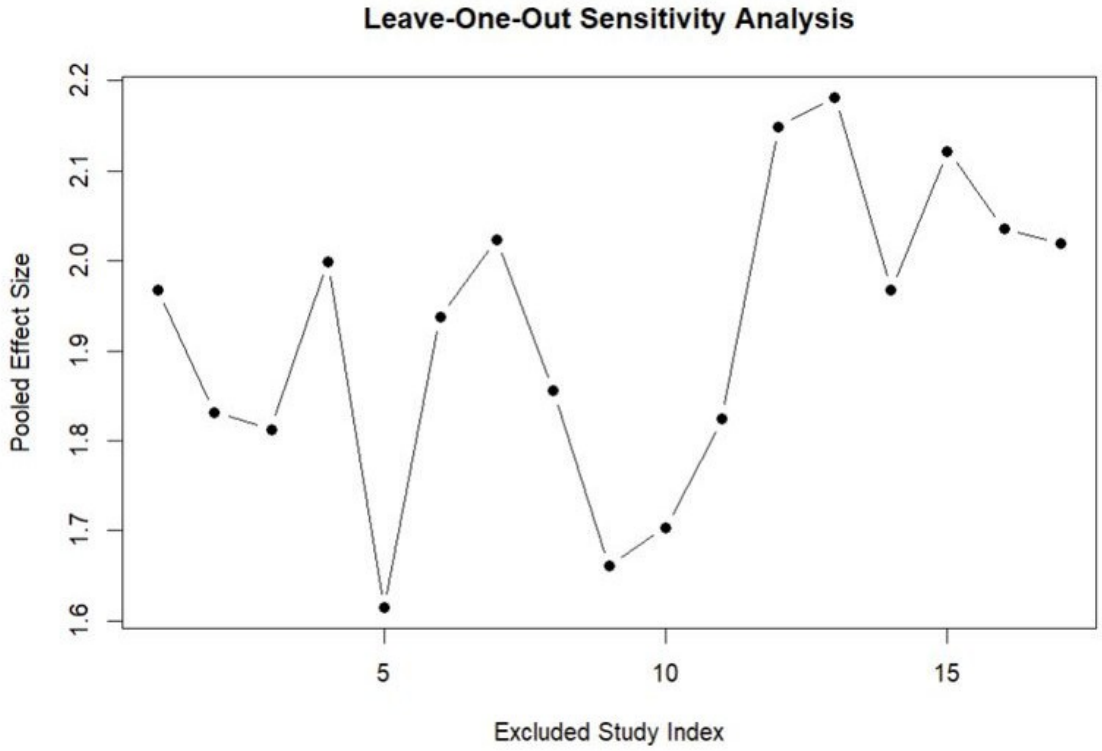

Figure 4. Graphical representation of leave-one-out sensitivity analysis, displaying effects of individual studies on overall effect size.

Meta-regression analysis

Meta-regression was employed to explore the impact of study-level controlled variables (covariates) on effect size variability. However, the covariate tested, log-transformed sample size (log(n_treatment)), did not significantly moderate the treatment effect (β = 0.2871, SE = 0.9562, z = 0.3003, p = 0.7640), and the model explained none of the observed heterogeneity (R^2 = 0.00%) (Figure 5). Taking this into account several subgroup analyses were performed.

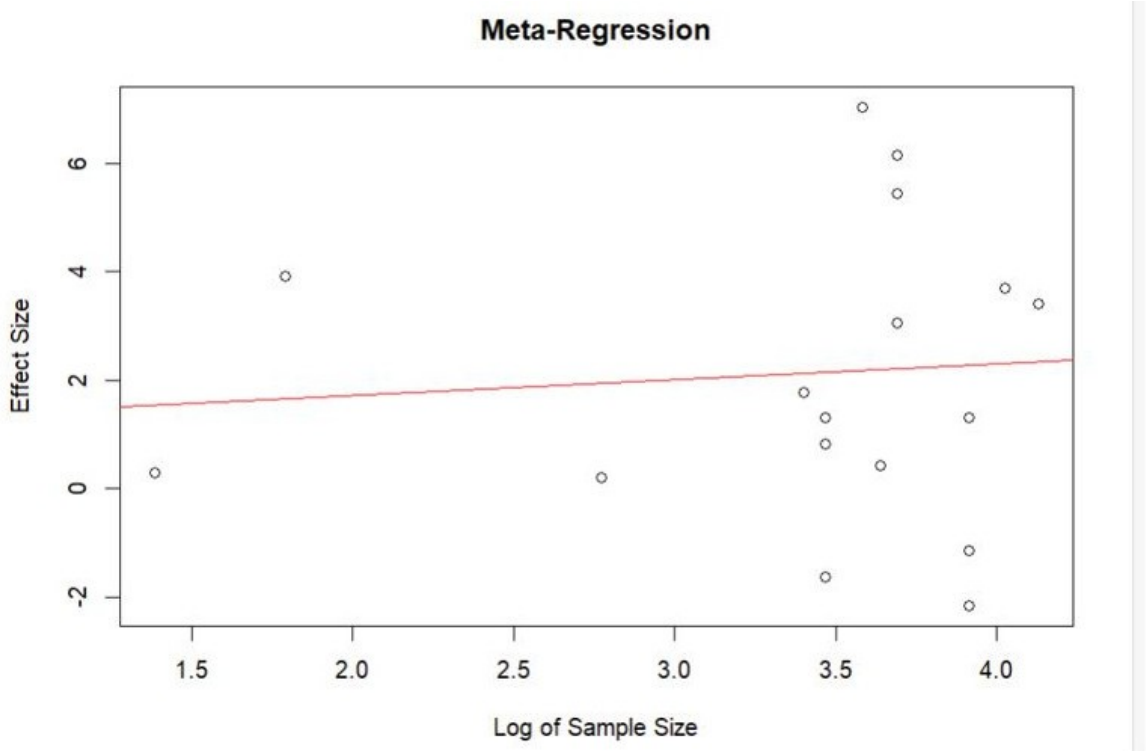

Figure 5. Graphical representation of meta-regression utilizing sample size as the covariate moderator to assess causes of heterogeneity.

Subgroup analysis

The subgroup analysis revealed significant differences in effect sizes between studies reported before 2020 and those reported in or after 2020. Studies reported in 2020 or later had a significantly higher effect size (estimate = 2.4423, SE = 0.9015, z = 2.7092, p = 0.0067) with a 95% confidence interval ranging from 0.6754 to 4.2092. Conversely, studies completed before 2020 showed a non-significant effect size (estimate = 1.3630, SE = 0.9394, z = 1.4510, p = 0.1468) with a 95% confidence interval from -0.4781 to 3.2042. The test of moderators further confirmed the significance of the year of study completion as a moderating factor on the reported effect sizes (QM = 9.4452 with 2 degrees of freedom, p = 0.0089), indicating a statistically significant impact of the timing of study completion on the treatment effects. These findings suggested that the timing of study completion was an important factor influencing reported effect sizes, with studies finalized in or after 2020 demonstrating higher effect sizes than those completed earlier. This may reflect evolving research methodologies, changes in treatment protocols, or differences in patient populations over time. Nonetheless, the substantial heterogeneity observed across studies indicated that other unmeasured; or unaccounted for factors also contributed to the variability in reported treatment effects.

Building on these findings, the significant level of heterogeneity observed across studies prompted a deeper investigation into potential sources of this variability. To this end, a second subgroup analysis was conducted based on the timing of outcome measurements, specifically distinguishing between studies that measured outcomes "Before 48 Weeks" and those that did so "48 Weeks and After." The mixed-effects model found a significant difference in effect sizes between the two subgroups. Studies measuring outcomes "Before 48 Weeks" demonstrated a significant positive effect size (estimate = 3.0628, SE = 0.5508, z = 5.5605, $p < .0001$) with a 95% confidence interval ranging from 1.9832 to 4.1424. This indicated a robust and statistically significant effect in this subgroup, suggesting that shorter-term outcomes following interventions were generally positive and significant in terms of dystrophin restoration. Conversely, for studies with outcome measurements "48 Weeks and After," the effect size was not significant (estimate = -0.9496, SE = 0.8457, z = -1.1229, p = 0.2615) and the 95% confidence interval ranged from -2.6071 to 0.7079. This interval crosses zero, indicating a lack of significant effect in the long-term outcomes subgroup. The negative direction of the effect size estimate also contrasted with the positive effects observed in the shorter-term outcomes, although this difference was not statistically significant.

The remaining heterogeneity within the model remained high ($I^2 = 94.28\%$), despite the subgroup categorization, suggesting that other factors might also contribute to the variability of effects across studies. However, the test for moderators (QM = 32.1799, $p < .0001$) indicated that the time point of outcome measurement significantly moderated the effect sizes observed, showcasing the importance of considering the timing of outcomes in evaluating intervention effects.

Conclusion

This meta-analysis provides an overall evaluation of exon skipping therapies for treating Duchenne Muscular Dystrophy (DMD), a severe genetic disorder characterized by rapid muscle degeneration due to insufficient dystrophin production (1,8). By focusing on the biochemical restoration of dystrophin measured through Western blot or the BL6 standard curve analysis, this study discovered several critical insights. Though several individual studies have explored exon skipping agents to treat DMD, they find a broad spectrum of effectiveness. This variability calls for a comprehensive meta-analysis that pools together these findings to display the overall trends and efficacy. While there does exist a meta-analysis that compiles such studies, its focus was primarily on physical and clinical outcomes rather than on molecular markers of exon skipping success, such as dystrophin expression. This represented a significant gap in the literature (20). This study addressed this crucial gap by concentrating on the quantification of dystrophin expression across diverse studies. By doing so, this meta-analysis brought forward a more nuanced understanding of the overall molecular impacts of exon skipping therapies, thereby advancing the potential for developing more effective treatments for DMD.

As shown, this analysis found a significant pooled effect size of 1.9237 (p=0.0028). Considering that an effect size over 0.8 is generally interpreted as indicative of a large intervention impact, the findings of this study suggest that exon skipping leads to a significant increase in dystrophin level, which may be directly linked to improved muscle function and stability in DMD patients (30). The clinical relevance of these findings cannot be overstated as even a partial level of dystrophin restoration can potentially slow the progression of muscle degeneration and extend the functional capabilities of patients, thereby improving their QoL (31-33).

However, the extremely high heterogeneity observed (I^2 = 97.2%) highlights the variability of effectiveness of exon skipping among patients. Through subgroup analysis this study found that the high level of heterogeneity could be attributed in part to the variability in the timing of dystrophin measurements, and the year in which the study results were completed. However, further research is needed to explore possible causes of the remaining heterogeneity (I^2 = 94.28%), including the difference in the exon targeted by the therapy, individual genetic profiles, and the stages of disease at which the treatment is initiated. These factors also highlight the complexity of DMD treatment and the necessity for a personalized medicine approach, where treatment strategies are more tailored to the specific needs of each patient (34).

The study sheds light on the significant advancements in exon skipping technologies, particularly those documented in studies published in or after 2020. These recent studies showed a higher efficacy of treatments (SMD=2.4423, p = 0.0067) compared to earlier studies. These time-related improvements suggested that ongoing research and technology advancements are enhancing the precision and effectiveness of these therapies. They are a promising indication that the field of medical science is evolving in the right direction, which may eventually lead to more standardized and effective treatments for all Duchenne Muscular Dystrophy patients (35).

Another critical insight from these findings was the apparent short term nature of treatment efficacy, with long term effects (48 weeks or greater) showing non-significance (estimate = -0.9496, p = 0.2615). This indicated that though exon skipping increases dystrophin level initially, maintaining these levels over a longer period of time might even require continuous or repeated interventions. This has significant implications for treatment planning, including a shift in clinical practice towards more long-term management strategies, emphasizing the importance of regular monitoring of dystrophin levels and faster adjustments to treatment protocols to try and maintain the efficacy of the treatment (32). However, the implications of this necessity extend beyond clinical settings, urging researchers to focus on developing therapies with prolonged effects to serve as one-time cures. Further, this also indicates the need for health policy to adapt to be able to support more sustained treatment plans, potentially even needing more long-term financial support for patients (36). This overall approach will be crucial in ensuring that advances in treatment translate into more sustainable improvements in patient outcomes.

A significant limitation of this research is the high heterogeneity observed and evidence of publication bias as evidenced by a semi-asymmetrical funnel plot, hence suggesting the need for a more transparent and consistent reporting style in clinical trials. Due to this, the results need to be interpreted with caution, as they may be inflated. Future individual exon skipping research in relation to DMD should focus on standardizing methodologies and outcome measures across studies to minimize the bias and improve the comparability of results (37). However, aside the inherent limitations stemming from the variability in results, this study design itself poses several noteworthy limitations. Due to funding restrictions, this study was limited to only include open-access, freely available articles. This forced selection of open-access articles represents a significant constraint, as numerous potentially relevant studies that may have met the rest of the eligibility criteria were excluded, potentially skewing the results and impacting the generalizability of these findings. Additionally, the assessment of dystrophin restoration was solely measured through quantified levels of Western blot bands. While this method provides a reliable measure of dystrophin expression, it does not fully capture other critical dimensions of protein functionally and distribution that methods such as immunofluorescence intensity or percent of dystrophin positive fibers restored might find. This may overlook nuances that are crucial for fully understanding the therapeutic impact of exon skipping in DMD. Such limitations in measurement coverage highlight the need for future research to approach measurement with a functionally broader repertoire of methods to ensure an overall portrayal of the treatment efficacy.

Moreover, the debate on the efficacy of dystrophin restoration as a surrogate endpoint introduces another layer of complexity to the interpretation of these results. Some studies argue that small increases in dystrophin may not lead to meaningful clinical improvements, potentially resulting in an overestimation of treatment efficacy (38,39). Conversely, even slight dystrophin restoration might significantly delay disease progression, indicating that the meta-analysis could have underestimated the treatment's benefits (38,39). This conflicting perspective suggested that while the pooled effect size indicated a signifiant increase in dystrophin, the true clinical impact might vary more widely than the data suggested. The evidence of publication bias and high heterogeneity observed in this meta-analysis suggested that the positive results reported in some studies may be inflated or not entirely representative of the broader population of DMD patients. The semi-asymmetrical funnel plot and significant Egger's test result indicated a potential bias towards publishing studies with favorable outcomes, which could skew the overall effect size reported. This may have led to an overestimation of the treatment's efficacy as the published literature may not have fully captured or accounted for less favorable or null results. Further, as the analysis revealed, while there was a significant initial increase in dystrophin levels (SMD = 1.9237, $p = 0.0028$), the lack of sustained long-term efficacy (estimate = -0.9496, $p = 0.2615$) underscored the limitations of using dystrophin levels as an endpoint without considering long-term clinical outcomes. Future research should aim to

address these limitations by incorporating more comprehensive and longitudinal measures of clinical efficacy to provide a clearer understanding of the true therapeutic potential of exon skipping therapies for DMD.

Despite these limitations, looking ahead, this research has significant implications for the broader medical community involved in the care of individuals with DMD. It highlights that continued research is essential to try and be able to optimize exon skipping strategies, including items such as improvements in delivery and dosage plans. More longitudinal, observational studies with extended follow up periods are important to be able to track the trajectory and efficacy of treatments over time, therefore informing clinical decision-making and facilitating the development of more personalized treatment plans created to the unique needs of the patients.

From a broader perspective, DMD displays the more overall challenges that are associated with rare diseases, such as limited treatment options, population heterogeneity, no significant correlation between relevant biomarkers and clinical outcome, delays in identifying the disease, and variations in access to care (40). Addressing these challenges requires a coordinated and more collaborative effort even at the international level, involving policymakers, healthcare providers, and researchers (36). By pooling resources, sharing knowledge, and combining efforts, future research can accelerate progress towards improved outcomes and ultimately allow exon skipping to serve as a cure for DMD and other rare genetic disorders.

Abbreviations

DMD: Duchenne Muscular Dystrophy, AONs: Antisense Oligonucleotides, QoL: Quality of Life

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Appendix. Studies used in the meta analysis

From Science Direct

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From clinical trials

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7. https://clinicaltrials.gov/study/NCT02420379?cond=Duchenne%20Muscular%20Dystrophy&intr=exon%20skipping&aggFilters=status:com&page=2&rank=12

8. https://clinicaltrials.gov/study/NCT02740972?cond=Duchenne%20Muscular%20Dystrophy&intr=exon%20skipping&aggFilters=status:com&page=2&rank=11

9. https://clinicaltrials.gov/study/NCT02310906?cond=Duchenne%20Muscular%20Dystrophy&intr=exon%20skipping&aggFilters=status:com&page=1&rank=6

10. https://clinicaltrials.gov/study/NCT02667483?cond=Duchenne%20Muscular%20Dystrophy&intr=exon%20skipping&aggFilters=status:com&page=1&rank=5

11. https://clinicaltrials.gov/study/NCT04179409?cond=Duchenne%20Muscular%20Dystrophy&intr=exon%20skipping&aggFilters=status:com&page=1&rank=1