Duchenne Muscular Dystrophy (DMD)

Subjects: Pediatrics

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Duchenne muscular dystrophy (DMD) is an X-linked recessive lethal disease that predominantly affects males, with an incidence of one case per 3500–9000 live births.

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1. Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive lethal disease that predominantly affects males, with an incidence of one case per 3500–9000 live births [1]. It is caused by mutations, insertions, or deletions of the *dystrophin/DMD* gene (location Xp21.2-p21.1) resulting in an absence of the cytoskeletal protein dystrophin. Dystrophin is an actin-binding protein that, through the α/β-dystroglycan complex, links the cytoskeleton to the extracellular matrix protein laminin, stabilizing the surface of muscle cells and preventing their rupture during contraction and relaxation cycles. Therefore, this is essential for the strength, stability, and functionality of the myofibers [2][3]. The absence of dystrophin in the muscle tissue causes a destabilization of the dystrophin-associated glycoprotein complex. This leads to, during contraction and relaxation cycles, sarcolemmal instability, and a decrease in force transmission by the sarcomere occurs. In dystrophic muscle there is an infiltration of mononuclear cells, variation in fiber size, centrally located nuclei, and degeneration and replacement of muscle tissue by fibrotic tissue. This leads to a progressive loss of muscle strength, due to the destruction of muscle tissue, along with the difficulty of muscle contraction of the remaining muscle, due to fibrotic tissue and muscle contractures. Ultimately, loss of ambulation (LoA) occurs in late childhood or early adolescence, in addition to other comorbidities such as cardiac and respiratory failure that reduce quality of life and life expectancy. Despite the optimization of glucocorticoid treatment and other pharmacological and nonpharmacological therapies, the prognosis remains poor [2][3][4][5][6][7][8][9]. However, some variability in the progression of DMD has been observed, even after considering improved treatments.

There are common genetic variants outside of the *dystrophin* gene and that are not pathological, which could influence disease progression through an anti-fibrotic or pro-fibrotic effect. The transforming growth factor beta (TGFβ) pathway is a complex signaling pathway that has been proposed as a candidate for modifying DMD progression, especially the age at LoA and cardiac remodeling. TGFβ is a cytokine that binds to the type II TGFβ receptor, which together with the type I TGFβ receptor forms a heterotetrameric complex, and the type I TGFβ receptor is phosphorylated at a region rich in glycine and serine residues, resulting in activation. Type I TGFβ then phosphorylates certain Smad proteins at C-terminal serine residues in a conserved C-terminal Ser-Ser-X-Ser motif. These phosphorylated Smads oligomerize with Smad4, and these complexes act on target genes, promoting homeostasis, cell differentiation, and tissue regeneration, among others [10]. At the muscle level, TGFβ1 and related cytokines, such as myostatin, a molecule belonging to the TGFβ superfamily, interfere with muscle growth and differentiation factors, such as myoblast determination protein 1 and insulin-like growth factor 2 [11]. This mechanism may also explain the cardiac remodeling that occurs with myocardial fibrosis [12][13].

In *mdx* models, plasma and muscle levels of TGFβ1 and the levels of TGFβ1 and connective tissue growth factor (CTGF) in the sarcoplasm of muscle cells and mesenteric interstitium are increased and correlate with the degree of fibrosis [14]. Two genes whose genetic variants might exert an effect on the phenotype are latent transforming growth factor beta binding protein 4 (*LTBP4*, location 19q13.2) and secreted phosphoprotein 1, also known as osteopontin (*SPP1*, location 4q22.1). LTBP4 binds to TGFβ, reducing its activity [15]. The homozygous IAAM haplotype (V194I, T787A, T820A, and T1140M) might create more stable binding, reducing TGFβ activity [16]. Meanwhile, *SPP1* stimulates TGFβ production and increased inflammatory cell infiltration [15]. Although in *mdx* models, *Spp1* genetic variants resulting in low basal activity are associated with an improved phenotype [17], the opposite result tends to occur in humans, showing the complexity of this pathway. Thus, for *SPP1*, the rs28357094 genetic variant, which is located in the *SPP1* promoter, has been proposed as a possible disease modifier [16].

In 2017, a systematic review suggested the association of some of these genetic variants with LoA [18]. Since then, new studies have been published that may improve the available evidence [19][20][21][22][23][24][25][26].

2. Main Findings

This systematic review and meta-analysis provides an overview of the evidence supporting the associations of genetic variants with LoA and cardiac function. Our results suggest a notable effect of genetic variants involved in the TGFβ pathway, especially *LTBP4* haplotype IAAM (in the recessive model), but not in patients who were exclusively treated with glucocorticoids, probably due to confounding factors. *SPP1* rs28357094 did not display a significant association. However, the use of glucocorticoids by patients carrying the *SPP1* rs28357094 genetic variant potentially increased the association in favor of genotype T (recessive model). Further research on *THBS1* genetic variants is needed, and the limited evidence available indicates an interaction with *LTBP4* that might improve the DMD phenotype. Finally, more research is needed on other proinflammatory and profibrotic pathways that might exert some effect and distort the effect observed in our study.

3. Interpretation

In dystrophic *mice* there is a 36-base-pair insertion/deletion site in a proline-rich domain of *Ltbp4*. The 12 amino acid insertion confers resistance to proteolysis of the TGFβ–LTBP4 complex, reducing TGFβ activity and therefore muscle fibrosis. Although this indel is not present in humans, the haplotypes mentioned in the previous sections (i.e., IAAM and VTTT) represent more than 80% of the total of all possible combinations in the human population. The haplotype IAAM behaves like the 36-base-pair insertion in *Ltbp4* of dystrophic mice, giving LTBP4 increased binding avidity to TGFβ, thereby reducing its activity [27]. Our meta-analysis shows that the *LTBP4* haplotype IAAM (or the rs10880 genotype T) is a protective factor resulting in prolonged ambulation. However, in patients treated exclusively with glucocorticoids, the haplotype IAAM had no effect, probably due to the inclusion of only two studies, one including patients from China, in which the *LTBP4* haplotype IAAM does not appear to be associated with LoA, perhaps due to the genetic/ethnic factors described below. Considering the Kaplan–Meier analyses, Barp A et al. and Chen M et al. did not observe an association, while Bello L et al. and Flanigan KM et al. reported a trend toward benefit for the haplotype IAAM, especially in the glucocorticoid-treated population. Interestingly, the cohorts in the former two studies (Barp A et al. and Chen M et al.) lost ambulation earlier than the cohorts in the latter two studies (Bello L et al. and Flanigan KM et al.), which might imply differences in the care and management or genetic/ethnicity of these patients. Thus, in the cohort analyzed by Barp A et al., the use of glucocorticoids was still not a universal standard, and a relatively low use of these drugs was reported. *Chen M* et al. investigated Asian cohorts, in which the effect of the *LTBP4* haplotype IAAM was different than that on Caucasian cohorts. Furthermore, previous evidence [27] has identified rs710160 as a genetic variant that may modulate the association obtained for the *LTBP4* haplotype IAAM with LoA. Thus, rs710160 genotype C together with the IAAM haplotype leads to less profibrotic signaling, potentially resulting in milder DMD. Additionally, rs710160 has a significant linkage disequilibrium with rs10880 in Caucasian populations but not in other populations, which might explain why the haplotype IAAM has a higher association with LoA in the Caucasian population but not in the Asian population, as described in the study by Chen M et al.

Thrombospondin-1, encoded by the *THBS1* gene (location 15q14), is a potent activator of the latent TGFβ complex. To activate TGF-β1, thrombospondin-1 interacts with the N-terminal region of the latency-associated protein, which binds non-covalently to TGF-β1. Thus, a trimolecular complex is formed, a conformational change occurs, and the reactivity of TGF-β1 is altered. TGF-β1 activation by thrombospondin-1 may be essential for the development of the heart, liver, bones, testes, and hematopoietic systems, among other tissues and organs [28]. Moreover, it exerts an anti-angiogenic effect by decreasing the activities of the nitric oxide and vascular endothelial growth factor (VEGF) pathway, and blocking endothelial cell migration, [28][29], which is harmful in animal models [30]. Finally, thrombospondin-1 could be useful in maintaining low but constant levels of active TGF-β1 [28]. In our study, *THBS1* rs2725797 genotype T is associated with lower thrombospondin-1 activity, with an additive effect with *LTBP4* rs10880 allele C, which may improve the phenotype of the disease, as suggested by the data presented in this entry.

SPP1 is a cytokine secreted by macrophages and myoblasts. It belongs to the family of small integrin-binding ligand N-linked glycoprotein secreted phosphoproteins and is expressed in numerous tissues in response to tissue damage/regeneration and inflammatory response. Alternative splicing and post-translational modifications make it a difficult cytokine to study. In *mdx* models without osteopontin, mice had reduced TGFβ levels, fibrosis, and increased strength, with reduced infiltration of inflammatory cells, neutrophils, and natural-killer T cells and increased numbers of regulatory T cells and M2 macrophages. Although the reduction in osteopontin could delay regeneration in acute damage, in mdx models (and probably in DMD) it decreases fibrosis caused by chronic damage [27][31]. Although in our study *SPP1* rs28357094 was not associated with LoA in a population that was not stratified by glucocorticoid use, in the glucocorticoid

subgroup, it appears to exert a glucocorticoid-dependent effect on LoA in regression analyses. In fact, in subgroup analyses stratified by glucocorticoid treatment, this genetic variant showed no association with LoA in the glucocorticoid-untreated subgroup or when a low percentage of glucocorticoid-treated patients was included. *SPP1* rs28357094 genotype G was detrimental to patients using glucocorticoids, with earlier LoA. Allele G is associated with lower osteopontin transcriptional activity under basal conditions [32]. However, *SPP1* likely contains glucocorticoid receptor elements, which modulate osteopontin expression through interactions with NF-kB, estrogens, and glucocorticoids. Thus, the *SPP1* rs28357094 genotype G might increase osteopontin expression by 3-fold in the presence of glucocorticoids, increasing profibrotic signaling [33]. *SPP1* rs11730582 might also use a similar mechanism. Genotype C, which results in higher initial osteopontin levels [34], is significantly associated with a later LoA in glucocorticoid-treated patients, suggesting that its effect is glucocorticoid-dependent, similar to rs28357094. Therefore, *SPP1* rs28357094 and perhaps rs11730582 are postulated to function not as disease modifiers but as predictors of a good or poor response to glucocorticoid treatment.

Regarding cardiac complications, no clear association with genetic variants can be observed due to the few included studies. *LTBP4* rs10880 genotype T tended to delay the age of DCM onset, with a significant association in patients treated with glucocorticoids [19]. This result is consistent with the data obtained for LoA. This association may be due to an additive effect of the *LTBP4* haplotype IAAM and glucocorticoids, but this requires further research. In contrast, another study did not detect an association of *LTBP4* with the development of left ventricular dysfunction. Interestingly, it was found that, among patients without left ventricular dysfunction, there was a higher proportion of patients with the rs10880 genotype T [25]. These discrepancies are, perhaps, due to the relatively small sample size, the age of the cohorts, or the categorization of patients into glucocorticoid-treated/untreated groups. Furthermore, the *SPP1* rs28357094 genotype T tended to be harmful in the development of DCM, including in the glucocorticoid subgroup [19]. This fact is interesting, since it is in the opposite direction to what happens in skeletal muscle. Conversely, it is in agreement with what has been observed in animal models, in which an overexpression of *Spp1* causes myocarditis and myocardial dilation [35]. Future research is needed in this regard. Cardiac complications, and especially heart failure, are one of the leading causes of death in patients with DMD. Both the *SPP1* genotype and glucocorticoids use could have some negative impact on the development of dilated cardiomyopathy. However, it should be noted that this does not necessarily imply progression to heart failure, and especially glucocorticoids could delay the onset of heart failure through other mechanisms.

The importance of the TGFβ pathway in the DMD phenotype may have several clinical implications: first, drug development aimed at the downregulation of TGFβ signaling to reduce muscle fibrosis. This approach includes angiotensin 1–7, halofuginone, anti-TGFβ1 antibodies, ixazomib, and angiotensin-II type 1 receptor blockers, which antagonize or downmodulate the TGFβ pathway with promising results in animal models [36][37][38][39][40]. However, some of these drugs have produced undesirable pleiotropic effects, which might be a problem in achieving a clinical benefit [38]. Another pathway is myostatin inhibition using compounds such as follistatin, ACE-031, domagrozumab, and the GDF11 propeptide that inhibit or antagonize the effect of myostatin [41][42][43][44][45][46]. However, the results from animal models and patients with DMD have raised some concerns related to other biological functions of myostatin that affect the metabolism and oxidative capacity of muscle fibers [41][47]. Second, genotyping of patients with DMD for *LTBP4* and *SPP1* could be considered, especially in the Caucasian population. Although knowledge of the genotype of *LTBP4* haplotype/rs10880 and *SPP1* rs28357094 would not alter the medical treatment of the patient, it can potentially provide the clinician and the patient and their family with more individualized prognosis as to their possible evolution and expected response to glucocorticoids. Third, genotyping can be considered in clinical trials of new drugs, such as treatments designed to restore dystrophin expression. Perhaps, by subgroup analysis according to genotype, part of the variability found in the results could be explained. It is possible that the patient's genotype can determine the response to small changes in dystrophin expression. Finally, the study of genetic variants in unknown DMD patients could be very interesting. Clinical DMD is well established, with diagnosis in early childhood. However, there are rare cases of dystrophinopathies, including Becker muscular dystrophy (BMD) and DMD, that remain undiagnosed until their debut in adolescence or adulthood, mainly due to cardiac involvement. [48][49][50][51][52]. Moreover, there are phenotypically intermediate forms of diagnosed dystrophinopathies, with slowly evolving DMD or rapidly evolving BMD [27]. These exceptional cases could be an opportunity to study the effect of genetic factors (known and unknown) as well as environmental factors on the progression of DMD and other dystrophinopathies.

4. Limitations

Some limitations should be acknowledged. First, the scarcity of studies included in the meta-analyses limits the statistical power and external validity of the results in larger cohorts of patients with DMD. Second, due to the limited number of studies, we were unable to perform publication bias, metaregression, or sensitivity analyses. This could question the

association for *LTBP4* and LoA. In systematic reviews, it is not uncommon for the first published studies to show stronger associations than subsequent ones, and they are even less likely to be published unless they question previous findings. Third, in some studies, the *LTBP4* haplotype was determined using a single SNP (mainly rs10880). Despite the large linkage disequilibrium, it could slightly underestimate the observed result. Fourth, in the Kaplan–Meier survival analysis, we were not always able to consider ethnic differences, the effects of other genetic variants, or a different proportion of glucocorticoid-treated patients, which would potentially modify the observed association. Fifth, participants who are candidates for exon 8 or 44 skipping tend to have slower disease progression [53][54]. Most studies did not consider this possible confounding genotype, and although it is possible that there are no statistically significant differences in the prevalence of these genotypes in the included studies, it cannot be ruled out, considering the ethnic and geographical diversity of the participants, overestimating or underestimating the effect observed for the genetic variants studied. Sixth, other genetic variants, such as *LTBP4* rs710160, and their possible differences in prevalence in different populations, might modify the association expected for the *LTBP4* haplotype IAAM. Seventh, some confidence intervals of hazard ratios were estimated from the *p*-value, which might differ from the true confidence interval. Eighth, in general, the authors did not determine the level of fibrosis of the patients' muscle tissue, which, in theory, should correlate negatively with LoA or cardiac function. This is probably due to the complexity of the procedures to obtain this information.

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