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## **The neurological and behavioral consequences of dystrophin deficiency in Duchenne muscular dystrophy: insights from mouse models**

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# Appendix

English summary

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Acknowledgements



## English summary

Duchenne muscular dystrophy (DMD) is a severe progressive disorder that affects both the muscles and the brain. The disease occurs in approximately 1 in 5,000 newborn males and is primarily characterized by severe muscle wasting, ultimately causing cardiac or respiratory failure. A subset of patients also suffers from brain related comorbidities, such as autism spectrum disorder, attention-deficit hyperactivity disorder, obsessive-compulsive disorder, anxiety, depression, dyslexia, dyscalculia and epilepsy. DMD is caused by mutations in the *DMD* gene, disrupting the production of dystrophin proteins. Healthy individuals have several dystrophin isoforms expressed in different tissues, each with their unique functions. Of these, Dp427, Dp140, Dp71 and Dp40 are present in the central nervous system. Depending on the location of the mutation, one or more dystrophin isoforms can be missing. Correlations have been found between the number of lacking brain isoforms and the incidence and severity of cognitive and behavioral problems. However, the exact function of the dystrophin isoforms, and the consequences of the lack thereof remains unknown.

Many animal models have been developed over the years to study DMD. These models have aided in understanding the disease and developing interventions for the muscle degeneration. However, for many of these models, especially those lacking multiple brain dystrophin isoforms, the cognitive and behavioral impairments have yet to be thoroughly investigated. Moreover, the absence of direct comparisons between models lacking different dystrophin isoforms limits our understanding of the precise consequences of missing the shorter dystrophin isoforms.

After a general introduction of DMD, the dystrophin protein and its functions in **chapter 1**, this thesis continued with an in-depth overview of the current knowledge on cognitive and behavioral deficits in DMD patients and different DMD mouse models in **chapter 2**. Here, we separated different behavioral domains, including, emotional reactivity, learning and memory and social interactions, and compared the known data from models lacking different dystrophin isoforms. We described the brain pathology in these models and summarized the current preclinical studies focusing on treating the DMD brain via exon skipping strategies to restore Dp427 and/or Dp140. While proof of concepts have been established, many challenges remain before the DMD brain can be successfully treated in humans.

In **chapter 3**, the behavior of two DMD mouse models was characterized and compared to each other. Here, *mdx* (lacking Dp427) and *mdx<sup>Δcv</sup>* (lacking Dp427 and Dp140) mice were subjected to behavioral tests to assess different types of learning, memory and spontaneous behavior. In addition, the blood-brain barrier was investigated via magnetic resonance imaging and immune-fluorescence staining. The deficits in working memory, movement patterns and blood-brain barrier integrity that were found in *mdx* mice, were also present in the *mdx<sup>Δcv</sup>* mice. The *mdx<sup>Δcv</sup>* mice

did not exhibit any additional deficits. Neither model displayed alterations in spatial learning or memory, learning flexibility, anxiety or other forms of spontaneous behavior, nor was expression of aquaporin 4 or glial fibrillary acidic protein (proteins associated with the blood-brain barrier) affected in either model. Overall, these results confirmed the involvement of Dp427 in working memory and blood-brain barrier integrity, as has been shown in literature before. This study further indicated that the lack of Dp140 does not have a significant impact on these processes.

**Chapter 4** continued the behavioral characterization of DMD mouse models, by using the *mdx<sup>5cv</sup>* (lacking Dp427), *mdx52* (lacking Dp427 and Dp140) and *DMD-null* (lacking all dystrophin isoforms) mouse models. A variety of behavioral domains was assessed, including anxiety, fear, learning and memory, social interaction and spontaneous behavior. Similar to the *mdx* mouse model, the lack of Dp427 in *mdx<sup>5cv</sup>* mice led to increases in anxiety and the fear response. The additional lack of Dp140 in *mdx52* mice appeared to have minimal impact on these processes; however absence of Dp71/Dp40 in *DMD-null* mice did further increase the already altered anxiety and fear response. *Mdx52* and *DMD-null* mice further showed subtle alterations in spatial memory during high motor demanding tasks, which was not seen in the *mdx<sup>5cv</sup>* mice. *Mdx52* mice also showed subtle changes in spontaneous behavior, mostly in habituation. More prominent alterations were observed in the *DMD-null* mice, which seemed restless and showed altered activity patterns due to the day/night rhythm compared to other models. Recognition memory was unaltered in the DMD mouse models. Unfortunately, no conclusions could be drawn on alterations in social interaction, due to a lack of social preference in our control group. Altogether, this study confirmed the role of Dp427 in emotional reactivity, and brought new deficits to light as a consequence of the lack of Dp140 and/or Dp71/Dp40, indicative of the cumulative effect the missing dystrophin isoforms have on behavior.

In **chapter 5** we aimed to investigate the effects of corticosteroids on behavior in DMD mouse models. Even though corticosteroids are part of the standards of care for DMD patients and these drugs are known to cross the blood-brain barrier, they are often overlooked in translational research. We implanted subcutaneous slow release pellets in *mdx* and *mdx<sup>4cv</sup>* mice, which were supposed to release prednisolone, or placebo, for 60 days. However, in the final stages of the study, it became apparent that the pellets were faulty and stopped releasing prednisolone approximately 3 weeks after implantation. During this 3 week active release window, animals underwent tests for anxiety, social interaction and spatial learning and memory. We found a small decrease in anxiety response in both *mdx* and *mdx<sup>4cv</sup>* mice that were treated with prednisolone compared to placebo treated animals. No differences were found in social interaction or spatial learning and memory. While we were unable to determine the long term effects of corticosteroid treatment in DMD mouse models, we showed that short term treatment does not have a negative effect on anxiety, social interaction or spatial learning and memory.



**Chapter 6** focused on the brain pathology in DMD mouse models. Here, *mdx<sup>5cv</sup>*, *mdx52* and *DMD-null* mice were used to assess and compare brain volumetrics, cerebral perfusion, cerebral diffusion and AQP4 expression. Mice lacking only Dp427 did not show any alterations. Lack of Dp427 and Dp140 led to a decrease in total brain volume, but not of that of individual brain regions. Surprisingly, mice lacking all dystrophin isoforms did not show the same decrease in brain volume as mice lacking Dp427 and Dp140. However, lack of all dystrophin isoforms did lead to reductions in cerebral perfusion and a complete disruption of AQP4 clustering. Interestingly, the lack of AQP4 clustering did not seem to affect cerebral diffusion, suggesting that other processes might have compensated for the disruptions found in AQP4.

**Chapter 7** contains a discussion of the implications of the findings described in this thesis and outlines the future directions of the field. It described the current challenges in the DMD field in terms of translatability and standardization in animal research and stressed the need for more inclusion of corticosteroids in preclinical DMD research, especially when investigating the brain. Lastly, the possibilities for treating the DMD brain are discussed, highlighting the long road ahead before we will be able to treat the DMD brain in humans.

DMD is a disease that involves many different processes and therefore both research and care should be of a multidisciplinary nature. The large impact of the cognitive and behavioral deficits on the quality of life of DMD patients and their families stresses the importance for more understanding of the brain involvement in DMD and the need for brain specific treatment. Although we still have a long road ahead before we might be able to treat the DMD brain, growing awareness and ongoing research offer hope for future breakthroughs that can enhance the lives of those affected by DMD.