A new web-based method for automated analysis of muscle histology

BACKGROUND: Duchenne Muscular Dystrophy is an inherited degenerative neuromuscular disease characterised by rapidly progressive muscle weakness. Currently, curative treatment is not available. Approaches for new treatments that improve muscle strength and quality of life depend on preclinical testing in animal models. The mdx mouse model is the most frequently used animal model for preclinical studies in muscular dystrophy research. Standardised pathology-relevant parameters of dystrophic muscle in mdx mice for histological analysis have been developed in international, collaborative efforts, but automation has not been accessible to most research groups. A standardised and mainly automated quantitative assessment of histopathological parameters in the mdx mouse model is desirable to allow an objective comparison between laboratories. METHODS: Immunological and histochemical reactions were used to obtain a double staining for fast and slow myosin. Additionally, fluorescence staining of the myofibre membranes allows defining the minimal Feret's diameter. The staining of myonuclei with the fluorescence dye bisbenzimide H was utilised to identify nuclei located internally within myofibres. Relevant structures were extracted from the image as single objects and assigned to different object classes using
web-based image analysis (MyoScan). Quantitative and morphometric data were analysed, e.g. the number of nuclei per fibre and minimal Feret's diameter in 6 month old wild-type C57BL/10 mice and mdx mice. RESULTS: In the current version of the module “MyoScan”, essential parameters for histologic analysis of muscle sections were implemented including the minimal Feret's diameter of the myofibres and the automated calculation of the percentage of internally nucleated myofibres. Morphometric data obtained in the present study were in good agreement with previously reported data in the literature and with data obtained from manual analysis. CONCLUSIONS: A standardised and mainly automated quantitative assessment of histopathological parameters in the mdx mouse model is now available. Automated analysis of histological parameters is more rapid and less time-consuming. Moreover, results are unbiased and more reliable. Efficacy of therapeutic interventions, e.g. within the scope of a drug screening or therapeutic exon skipping, can be monitored. The automatic analysis system MyoScan used in this study is not limited exclusively to dystrophin-deficient mice but also represents a useful tool for applications in the research of other dystrophic pathologies, various other skeletal muscle diseases and degenerative neuromuscular disorders.

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