that MPCs will receive mechanical tension without collagen VI. Thus, this analysis unraveled the molecular consequences in MPCs induced by the defect of collagen VI in skeletal muscles.

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Automated diagnosis of collagen VI related muscular dystrophies using advanced image analysis and machine learning

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It is becoming increasingly important to have quantitative methods not only to offer a precise diagnosis to patients but to monitor the efficacy of novel therapies and to study genetic variants of unknown significance. To this aim we quantified collagen VI on confocal images with an Image J based protocol, where we used integrated intensity normalized by the number of nuclei per field as the main feature of interest in fibroblast cultures. Using this method, we were able to detect a range of quantitative changes in the collagen VI matrix. However, given that in many cases collagen VI intensity was not affected, we developed a computer aided diagnostic system based on a Convolutional Neural Network (CNN) for the automated analysis of structural alterations in collagen VI extracellular matrix. The CNN was trained using 56320 image patches from cases labeled by specialists as controls or patients. Then, the system was tested using 14336 patches. Given an input image from a potential patient, it is split in patches, each patch is colored according to its probability of belonging to the control class as computed by the CNN, and a majority voting scheme is used take a decision. The system also gives an overall score computed as the percentage of patches classified as controls. The results of the initial test have demonstrated the robustness of the system. At the level of patch classification, the system achieved an accuracy of 0.95, a precision of 0.92, a recall of 0.98 and a F1 score of 0.95. At the level of images, the system achieved perfect classification of results (e.g. all the above metrics being 1). Furthermore, we analyzed the CNN to find out which were the key features used for the classification, resulting in the fibroblasts nuclei and the areas without collagen VI being the most relevant factors in the classification task. We conclude that these are feasible and sensitive methods for the assessment of collagen VI related muscular dystrophies

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Genome and transcriptome analysis of COLVI genes and characterization of a new promising cellular model

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Collagen VI-related diseases (COL6-RD) are a group of inherited myopathies with varying degree of clinical severity, caused by mutations in the COL6A genes. As EU reference center for neuromuscular disorders and partner within the EURO-NMD, we aim at providing a nationwide study of COL6-RDs patients and an overview of COL6A genes variants. 245 patients were recruited via our genetic counselling service as well as referred to us by other Italian centers and analyzed over a 12-year period (2006-2018). 222 patients were studied by standard diagnostic tools and 23 by NGS Illumina TruSeq Custom COL6A genes panel. 186 disease-causing variants, evenly distributed through the three COL6A genes, were identified in 150 patients with a detection rate of 61.3%. Using RNA tools (Custom FluiCol6 microfluidic card and RNA-Seq) we characterized the COL6A genes transcripts on urine stem cells (USCs) and fibroblasts from patients and healthy controls. The transcripts comparison with the skeletal muscle showed that some splicing choices and isoforms representation are different in USCs. Indeed, the COL6A3 full-length isoform represents the prevalent transcript in skeletal muscle while is expressed in USCs and fibroblast at very low levels. We also demonstrated that native USCs secrete functional collagen VI proteins, able to organize in the extracellular network. Our data provide a large COL6-RD patients cohort fully genetically characterized and propose native USCs as a non-invasive *in vitro* tool for functional studies, drug screening and validation in COL6-RDs.

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A novel *in situ* hybridisation (ISH) assay mapping the in-frame pseudoexon 11 (pE11) expression in cultured dermal fibroblasts (CDF) and skeletal muscle in patients with severe collagen VI disease due to a deep intronic mutation in *COL6A1*

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A recurrent, de-novo deep intronic mutation (intron 11) of *COL6A1* (c.930 + 189C>T) leading to a dominantly acting in-frame pseudoexon insertion was recently identified in patients with a severe collagen VI-related disease (COL6-RD) and represents the most common COL6 mutation. cDNA analysis showed lower expression of pE11-containing transcripts in muscle and CDF compared to wild type (WT) transcripts, despite the clinical severity and markedly reduced/mislocalised collagen VI, indicating a strong dominant-negative effect. Our aim was to develop an ISH assay for spatial and quantitative mapping of pE11-containing and WT transcripts and study effects of the mutation on collagen VI assembly in muscle and CDF. Highly specific short-length exon-junction mRNA BaseScope probes were custom-designed targeting E9E10 (total transcripts), E11pE11 (pseudoexon-containing transcripts) and E11E12 (WT transcripts) for brightfield detection. Immunoanalysis in muscle and CDF from 4 patients carrying the intronic mutation and unaffected controls (CTRL) were performed with chain-specific and multimeric collagen VI antibodies. In CDF from all 4 patients we observed uniform, low-level cytoplasmic expression of mutant pE11-containing transcripts with much higher expression of WT transcripts. CDF showed mildly reduced and qualitative abnormal extracellular collagen matrix in two cases, as well as variable intracellular retention of collagen in all four cases. The in-situ expression levels of mutant and WT transcripts, and immunoanalysis in CDF correlated with *COL6A1* RT-qPCR transcription data and quantitative flow cytometry results respectively. Digital analysis of muscle ISH data and high resolution imaging of the collagen VI matrix in CDF are in progress. These techniques could be useful tools for studying effects of dominant negative mutations on collagen VI assembly in COL6-RD, and the effect of therapies aimed at removing the pseudoexon from the mature *COL6A1* transcript

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