Alterated TDP43-Dependent Splicing in Sporadic Inclusion Body Myositis

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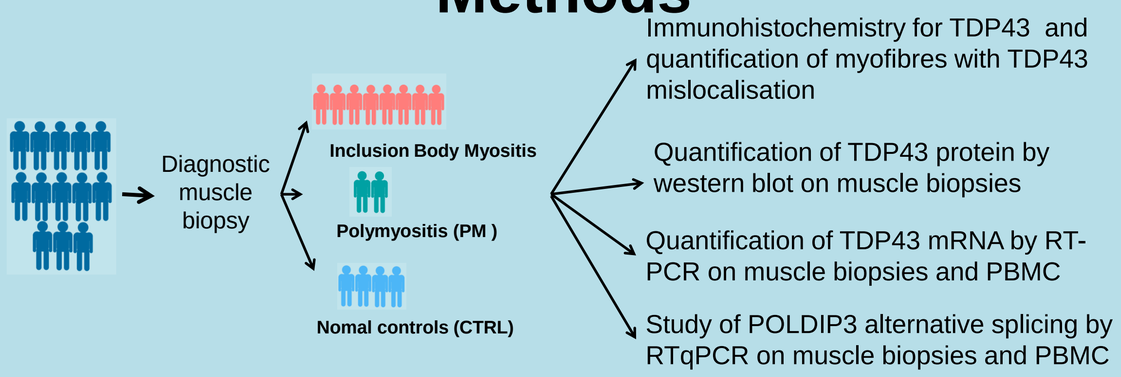
Introduction

Sporadic inclusion body myositis (sIBM) is the most common acquired myopathy occurring in adults aged older than 50 years. Abnormal cytoplasmic accumulations of TDP43, a prevalently nuclear RNA binding protein involved in numerous aspects of RNA metabolism, have been consistently described in sIBM myofibers. In a previous study we found in sIBM widespread changes of the expression and cytoplasmic mislocalization of numerous RNA binding proteins, including TDP43. However it is not known whether aggregation and mislocalization of TDP43 in sIBM are associated with changes in TDP43 function, bearing possible pathogenic relevance to muscle fibre degeneration.

Aim of the study

- To study the role of TDP43 in terms of quantitative and functional changes, measuring TDP43 mRNA expression and POLDIP3 alternative splicing, as a marker of TDP43 function in **muscles biopsies**
- To assess the tissue specificity of TDP43 quantitative and functional changes, measuring TDP43 RNA expression with RT-qPCR and analysing POLDIP3 alternative splicing in **peripheral blood mononuclear cells** (PBMC) as a non affected tissue

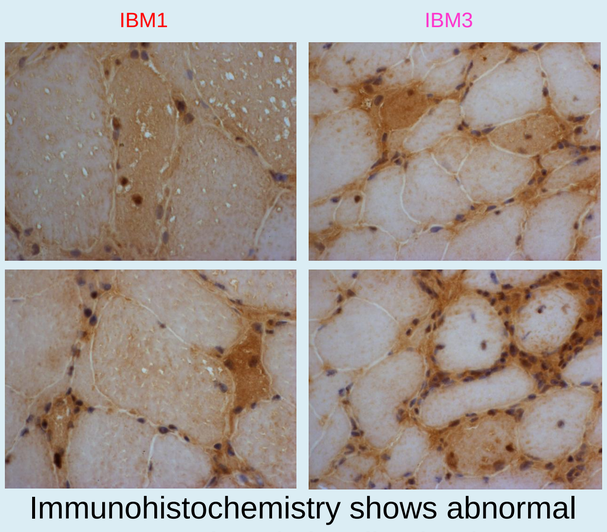
Methods



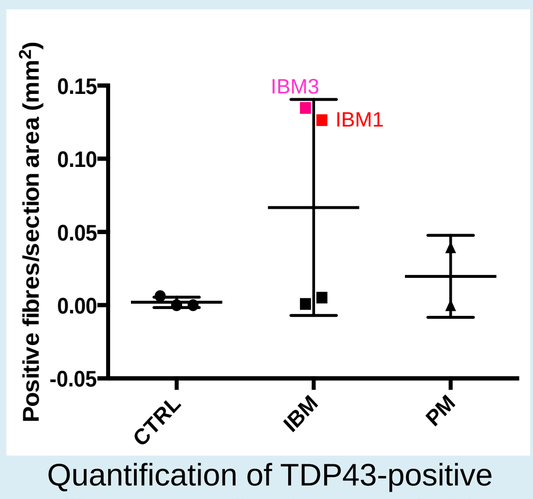
	sIBM	PM	CTRL
N	4	2	3
Gender (male)	3 (75%)	2 (100%)	2 (67%)
Age at biopsy (years)	63 ± 9 (56, 76)	35 ± 26 (27, 54)	44 ± 15 (22,54)
Site of biopsy			
Quadriceps femoris	3 (75%)	2 (100%)	2 (67%)
Biceps brachii	1 (25%)		1 (33%)
Disease duration (years)	8 ± 4 (5, 13)	1	-

Results

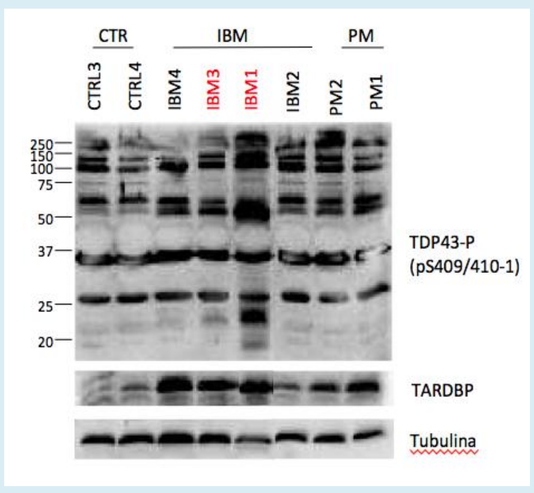
TDP43 mislocalize and accumulate in IBM

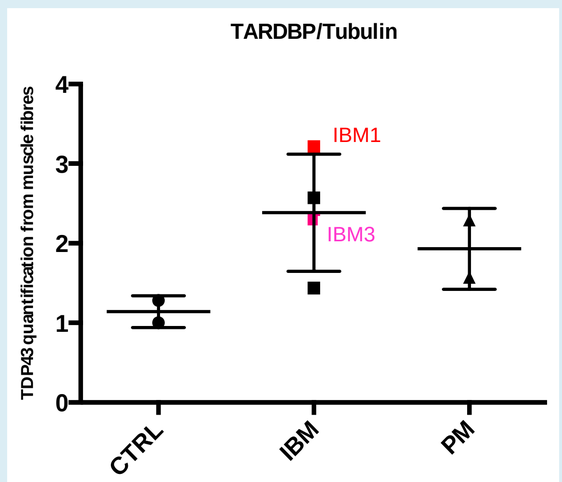


Immunohistochemistry shows abnormal TDP43 cytoplasmic localization in sIBM myofibers



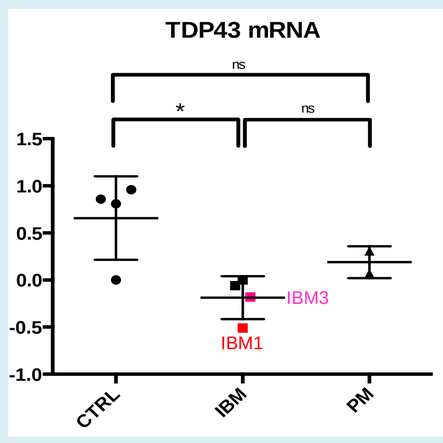
Quantification of TDP43-positive myofibres normalized to muscle biopsy area





Western blot for TDP43 and phosphorilated TDP43 shows higher amount TDP43 and phosphorylated TDP43 in IBM as compared to PM and CTRL

Levels of TDP43 mRNA from sIBM samples are lower than CTRL and PM

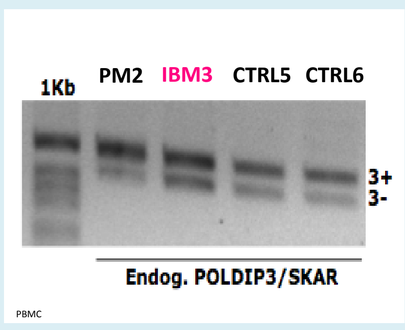


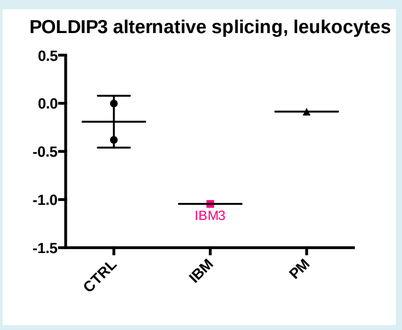
Altered TDP43-dependent POLDIP3 alternative splicing in IBM myofibres

Significant decrease of inclusion of POLDIP3 exon 3 in muscle fibres from sIBM patients compared to CTRL and PM

Note that sIBM samples (IBM1, IBM3) with higher amount of TDP43 protein accumulation, shows lower levels of TDP43 mRNA and higher amount of alternatively spliced POLDIP3 transcript.

Changes of alternative splicing of POLDIP3 are observed also in PBMC, although less prominent than in muscle fibres





Discussion

Here we demonstrate reduced level of TDP43 expression in muscles biopsies and PBCM of sIBM patients, providing, to our knowledge, the first experimental evidence of an altered TDP43 functionality in sIBM. Moreover we found an impaired function of TDP43 in muscles biopsies as well as in PBMC from sIBM, as demonstrated by altered levels of POLDIP3 exon 3 splicing. The higher extent of quantitative and functional changes in muscle fibres compared to PBMC points to a tissue-specific alteration of splicing processes, carrying a TDP43 signature. Whether other genes involved in viability of muscle fibres may be misspliced in sIBM due to TDP43 depletion and misfunction remains to be defined in future studies. However the limited number of muscles and PBMC samples makes this results only preliminary.

References

A. Cortese, V. Plagnolf, S. Brady, R et al. Widespread RNA metabolism impairment in sporadic inclusion body myositis TDP43-proteinopathy, Neurobiology of Aging (2014);35:1491-1498

Emanuele Buratti, Maurizio Romano, Francisco E. Baralle, TDP-43 high throughput screening analyses in neurodegeneration. Advantages and pitfalls, Molecular and Cellular Neuroscience (2014);56:465-474