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UMONS RESEARCH INSITUTE FOR HEALTH SCIENCES AND TECHNOLOGY

Development of easy outcome measures for the *in vivo* screening of therapeutical agents targeting DUX4 in FSHD

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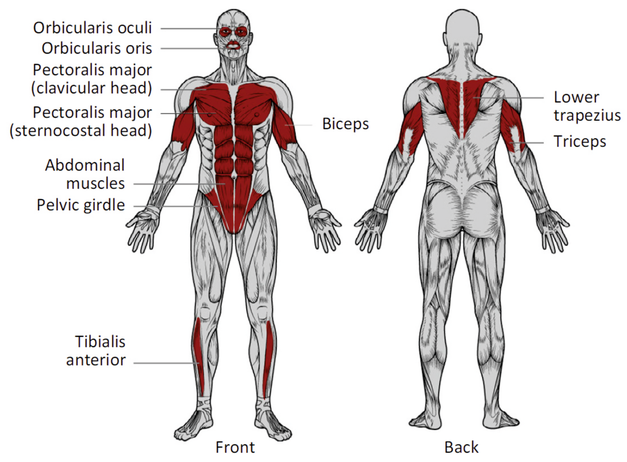
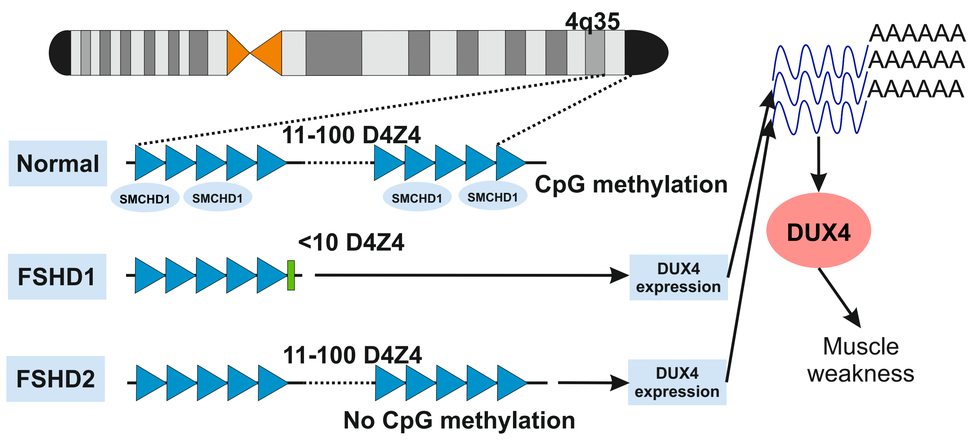
INTRODUCTION

Facio-Scapulo-Humeral Dystrophy

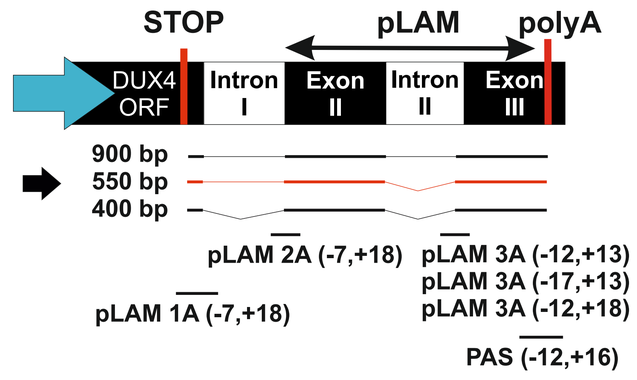


Lek et al., Trends Mol Med. 2015

Genetic and epigenetic background



Therapeutic strategy



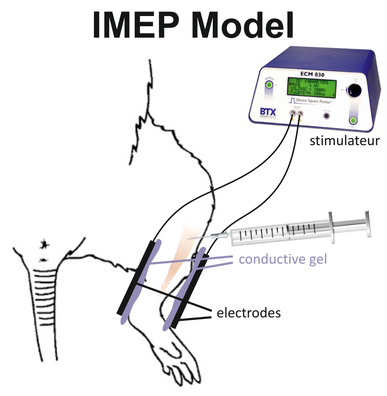
DUX4 mRNA variants found in FSHD myoblasts and positions of the antisense oligonucleotides (Aos) directed against *DUX4* mRNA

AIM

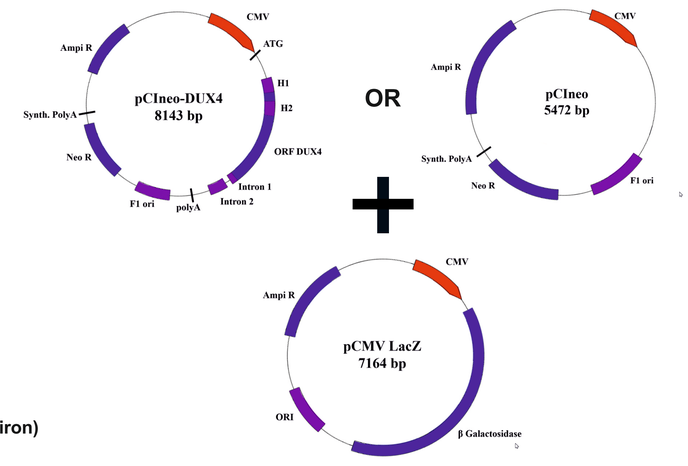
FSHD is a genetic disease with, currently, no definitive treatment. However, **antisens tools** designed against DUX4 transcripts have been developed and showed efficiency *in vitro*. These results have now to be confirmed in an *in vivo* model. Therefore, we have developed a **murine model** based on an intramuscular injection of a DUX4 expression vector followed by an electroporation (**IMEP**).

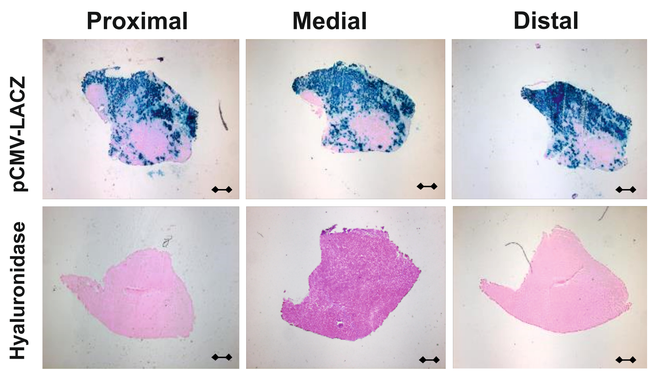
IN VIVO MODEL

IMEP Model



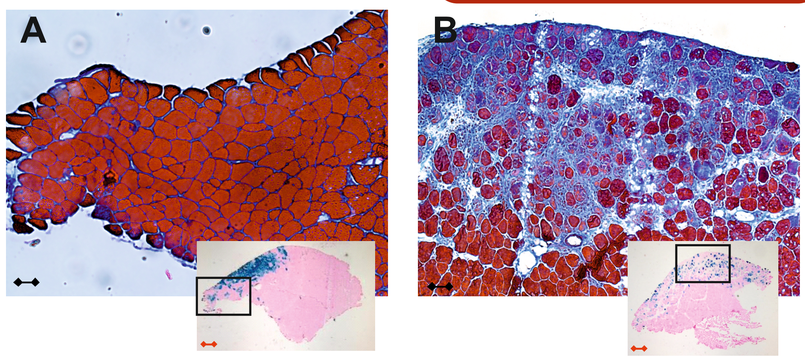
Modified from Hagstrom et al., Mol. Ther. 2004 (B. Blairon)
Method described in Vilquin et al., Gene Ther. 2001





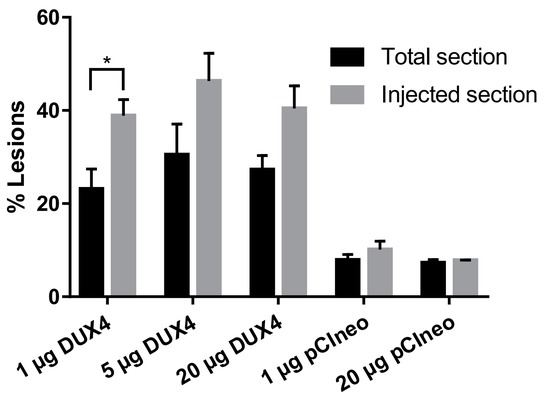
Optimization of the IMEP method using a LACZ reporter vector. 40 µg of *pCMV-LACZ* (encoding for ß-galactosidase) or 50 µl of saline solution were injected intramuscularly in the tibialis cranialis (TA). This procedure was followed by an electroporation (IMEP). One week after injection, muscles were harvested and cryosections were stained with X-Gal to assess LACZ+ areas. Scale = 500µm.

HISTOLOGY



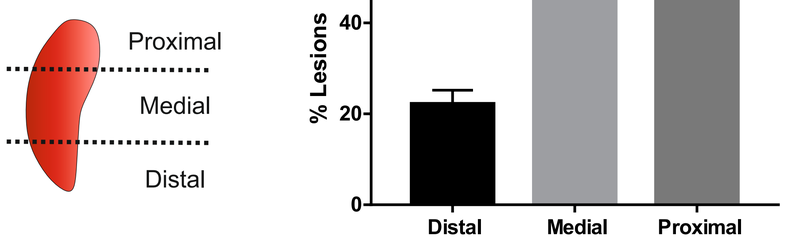
DUX4 IMEP model: TA muscle morphology. Mouse TA was co-injected by IMEP with *pCMV-LACZ* and *pCIneo* **(A)** or *pCIneo-DUX4* **(B)** plasmids. One week after injection, muscles were harvested and cryosections were stained with X-Gal to assess LACZ+ areas. Adjacent sections were coloured with HEB. Scale = 500µm. Scale = 100µm.

DOSE RESPONSE



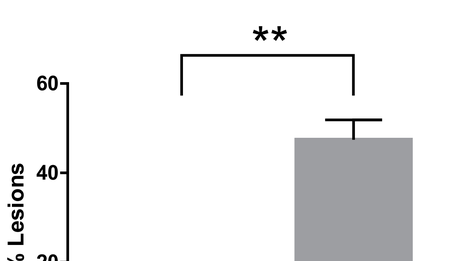
Dose-response analysis. Quantification of damaged surface areas were performed on Total/Injected sections using HEB coloration and colour thresholding.T-test , *p<0,05, n=3.

MUSCLE REGION COMPARISON



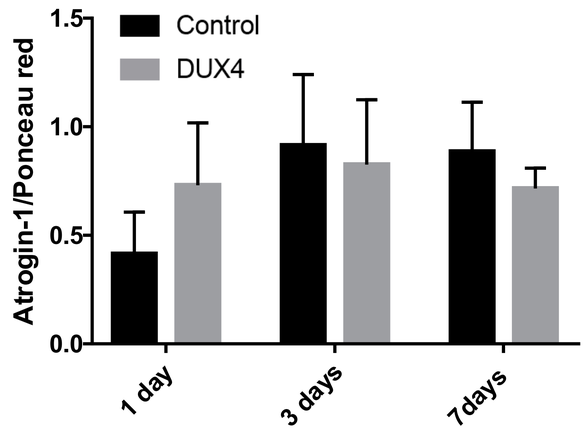
Lesion distribution through TA regions. One way ANOVA, *** p<0,001, n=3.

TIME-POINT COMPARISON

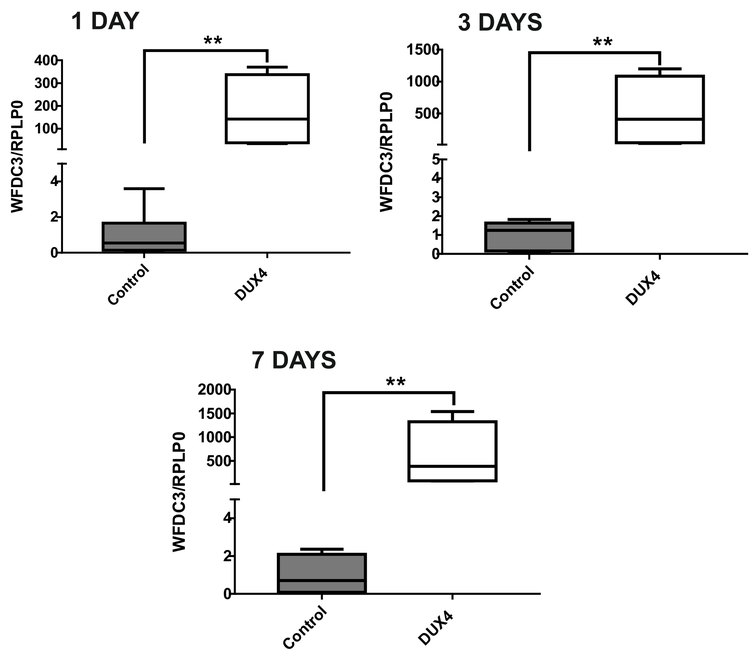


Time-course analysis. TA muscles received 1µg of *pCIneo-DUX4*. T-test, **p<0,01, n=3.

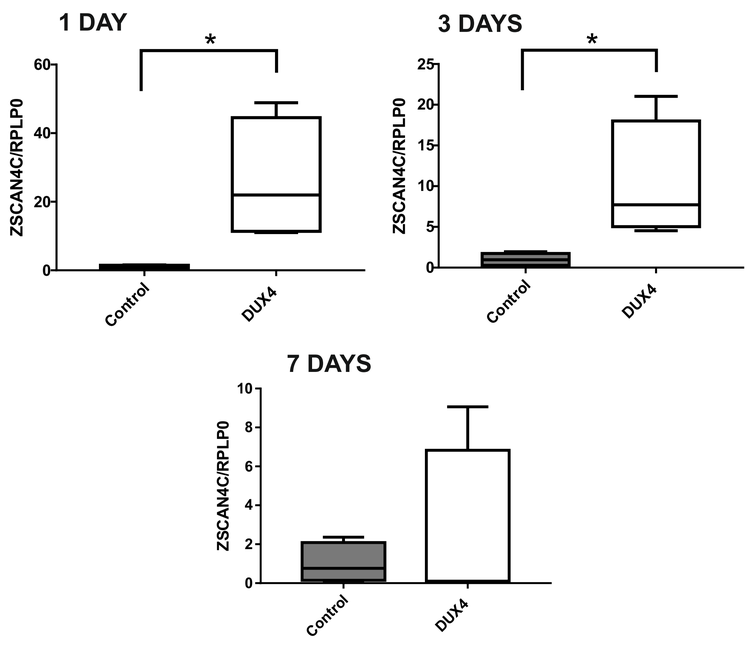
DEREGULATED GENES



Relative quantification of atrogin-1 protein level in IMEP model (SDS-PAGE followed by a Western Blot). One-Way ANOVA, n=3, NS.

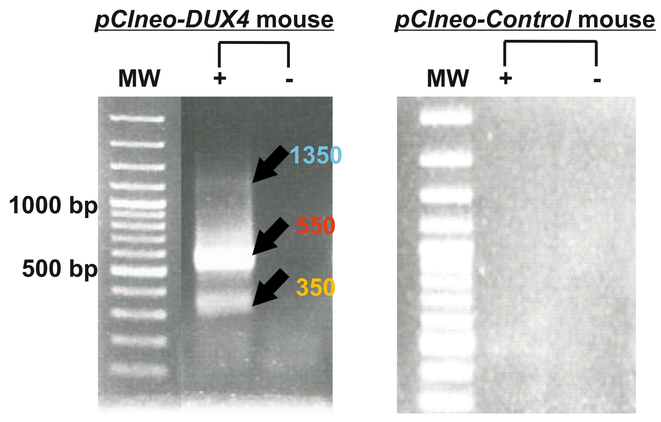


WFDC3 expression in IMEP model (RT-qPCR). Mann-Whitney Rank Sum Test, **p<0,01, n=4 for DUX4 and n=8 for Control.

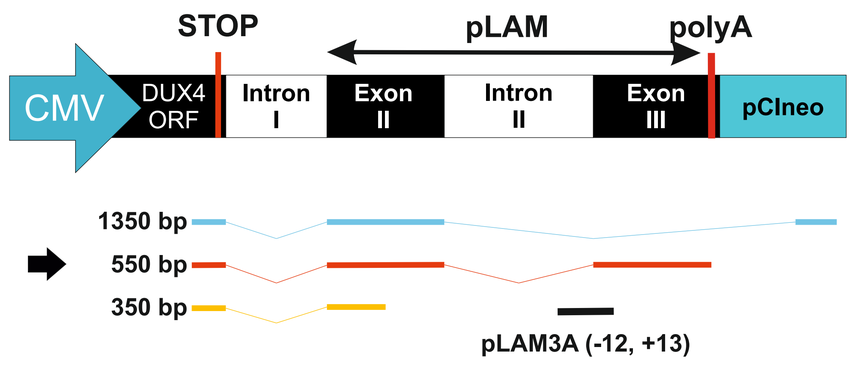


ZSCAN4c expression in IMEP model (RT-qPCR). Mann-Whitney Rank Sum Test, *p<0,05, n=4.

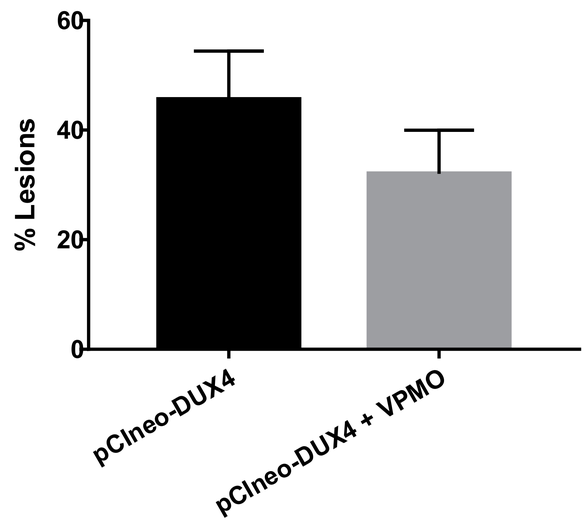
DUX4 SILENCING



3'RACE products from DUX4 and control IMEP mouse model. Arrows show 1350, 550 et 350 bp fragments. Wells (-) show negative control without retrotranscription. 3'RACE were performed after 1, 3 and 7 days after injection.



Schematic representation of 3'RACE products sequence analyses detected in IMEP model.



Quantification of TA muscle damages after vPMO treatment in IMEP model. TA muscle was injected with 5 µg of *pCIneo-DUX4* and either 10 µg control vPMO or pLAM 3A (-12, +13) vPMO. Means were calculated with data from medial and proximal regions (n=1).

CONCLUSION

We have established a **murine model** based on an intramuscular injection of a **DUX4 expression** vector followed by an electroporation in TA muscle . Atrophic muscle fibres, inflammatory infiltrates and expansion of extracellular matrix were observed. **Damaged surface areas** were quantified using HEB staining and colour thresholding. Induction of **DUX4 deregulated genes** were robustly detected. ***DUX4* mRNA** was studied by 3'RACE and three fragments have been detected. The most abundant fragment was previously observed in FSHD human muscle cells and carries the **target sequence** of available **antisens tools** (*Vanderplanck et al, 2011*). This indicates that the IMEP murine model can be used to **screen therapeutical agents** targeting DUX4.

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