FKRP related Limb Girdle Muscular Dystrophy:
A biomarker identification study

Evelyne Gicquel[1], Camille Vaubourg[1] and Isabelle Richard[1]

1: *Généthon, INSERM U951 INTEGRARE, Evry, F-91002, France*

Introduction

LGMD-R9 is due to mutations in the ***FKRP*** gene, encoding the **Fukutin Related Protein**.

FKRP is involved in α-dystroglycan (αDG) glycosylation:
In this complex multi-step process, the precise function of FKRP is the addition of a ribitol-5-phosphate in the sugar chain being formed, in the Golgi apparatus.
Glycosylation defects of αDG disrupt its proper binding to extra-cellular matrix (ECM) components, and therefore the link between the cytoskeleton and the ECM. Muscle fibers become less resistant to muscle contractions.

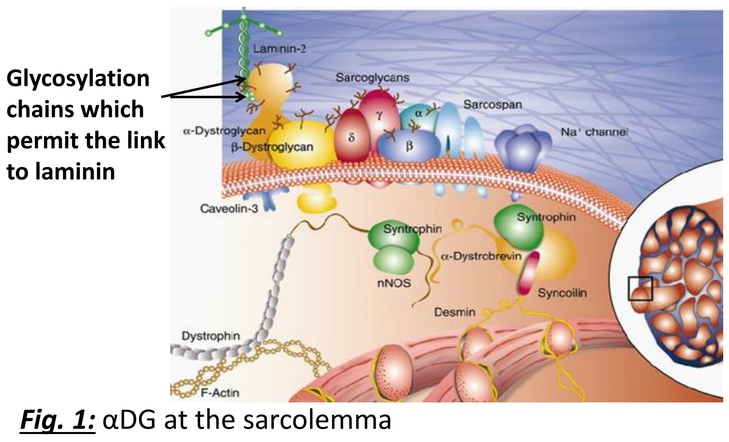
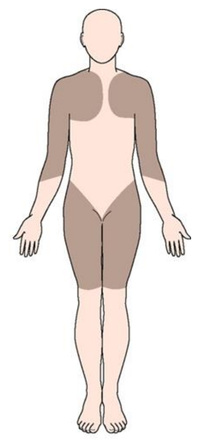


Fig. 1: αDG at the sarcolemma
(from J Ehmsen, E Poon and K Davies)

LGMD-R9 characteristics:
- Affected muscles located at scapular and pelvic girdles;
- One of the most frequent LGMDs in Europe.



Preclinical studies showed the efficacy of FKRP gene transfer

-> clinical trial in preparation

Objective of the study : to identify LGMD-R9 biomarkers in natural fluids (blood and urine)

Cohort: 24 LGMD-R9 patients (24 women / 3 men) and 9 healthy donors (6 women / 3 men).

Study of protein biomarkers – candidate approach

Myomesin 3 fragments in serum
Described as biomarker for DMD
(*Rouillon et al., 2015*)

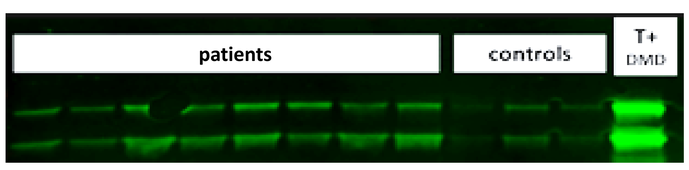
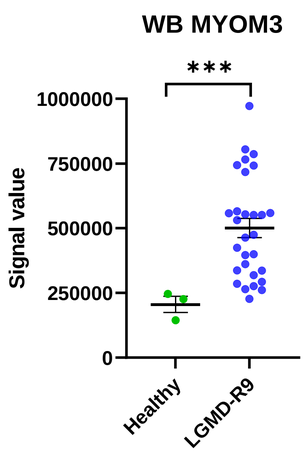


Fig2:
Myomesin 3 fragments detection and quantification, by western-blot.



Titin 25 kDa fragment in urine
Described as biomarker for DMD
(*Rouillon et al., 2014*)

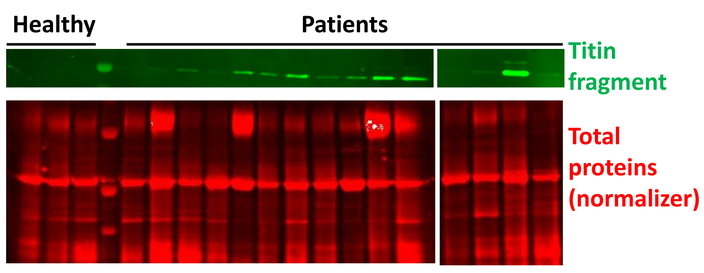
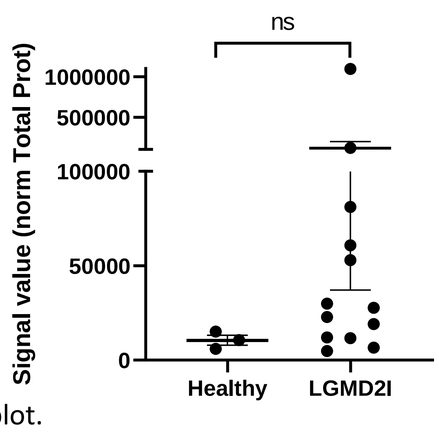


Fig3:
Titin fragments detection and quantification, by western-blot.



⇒ **Myomesin 3 fragments are present in blood samples of LGMD-R9 patients. The 25 kDa titin fragment is present in urine with high variability.**

Study of circulating micro-RNAs – global approach

Seric miRNAs high throughput sequencing
Many miRNAs were found different between patients and healthy controls, with specific profiles for women and men.

Women: 179 miRNAs
Men: 40 miRNAs | *7 in common*

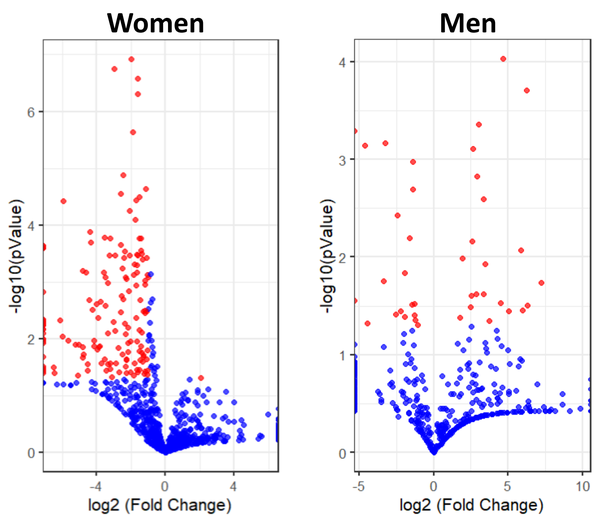


Fig4:
Volcano plots presenting the results of miRNA sequencing in the human cohort serum samples.

In mice (males only)
miRNA sequencing of FKRP-deficient mice.
4 months old, n=4

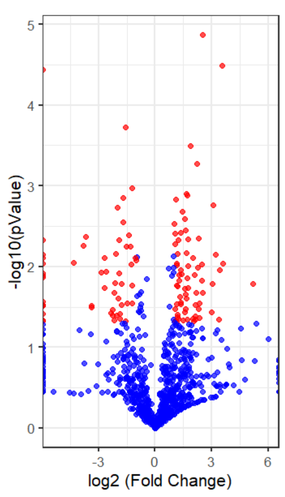


Fig5:
Volcano plot presenting the results of miRNA sequencing in FKRP-deficient mice serums.

From top-15 miRNA list in men:
-> 5 are also dysregulated in male mice.

Comparison with other MD mouse models
Some of the selected miRNAs are markers of several MDs, some others are more specific to LGMD-R9.

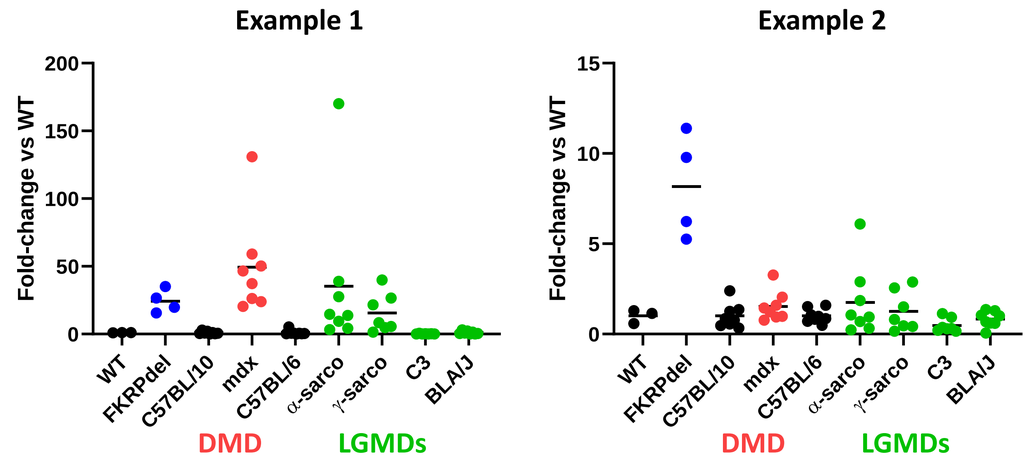


Fig6: Quantification of miRNAs by qRT-PCR, in different mouse models serums.

Restoration after FKRP gene transfer
The effect of the AAV-FKRP is noticeable on most of the selected miRNAs.

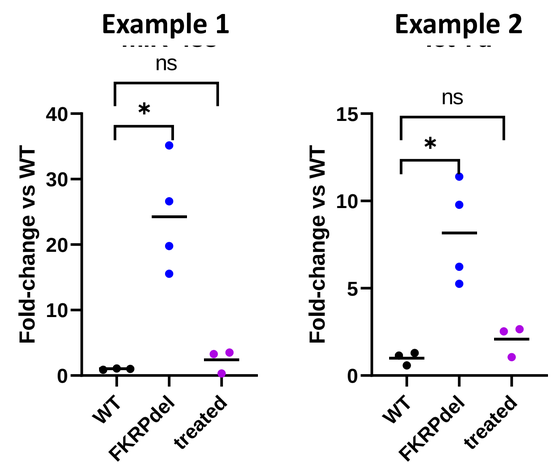


Fig7: Quantification of miRNAs by qRT-PCR, after FKRP gene transfer.

⇒ **Circulating miRNAs are also good candidates as LGMD-R9 biomarkers.**

Conclusions

Using samples from a cohort of LGMD-R9 patients, we identified several types of biomarkers that could be useful for the monitoring of LGMD-R9 pathology and treatment. Myomesin 3 fragments in blood and titin fragments in urine are easily detectable protein biomarkers. Micro-RNAs sequencing in serum led to the selection of a panel of circulating micro-RNAs that discriminate patients from controls. These new tools will be useful for patients follow-up in the upcoming gene therapy clinical trial.