Multi-approach study of *Pla2g4e* involvement in obesity development in unique fat and lean mouse lines

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INTRODUCTION

Obesity is controlled by a combined effect of genetic and environmental factors. The mechanisms of action that trigger metabolic disorders have not been fully discovered yet.

In our study, we used the Fat (F) and Lean (L) mouse lines representing unique polygenic models of obesity and leanness (Fig. 1). Previous transcriptome studies on these lines fed standard maintenance chow diet identified gene *Pla2g4e* as differentially expressed in hypothalamus and muscle tissue. Pla2g4e is involved in nutrient metabolism and energy expenditure. It is highly expressed in the skeletal muscles and is involved in their metabolism and function.

Our experiment aimed to study *Pla2g4e* on RNA and protein levels comparing F and L mouse lines.

Fig. 1: Our unique mouse models for studying leanness and obesity. L - Lean line, F - Fat line.

METHODS

Mice of F and L lines of both sexes were fed two different isocaloric diets from weaning to the age of 14 weeks:
- high-fat diet (HFD) or
- low-fat diet (LFD)

Mouse body weight and feed intake were monitored weekly.

Mice were sacrificed at the age of 14 weeks and skeletal muscle tissue was collected and frozen at -80 °C.

Musculus gastrocnemius:
- RNA isolation
- *Pla2g4e* mRNA expression analysis by quantitative real-time PCR method (house-keeping genes *Gapdh* and *Actb)*

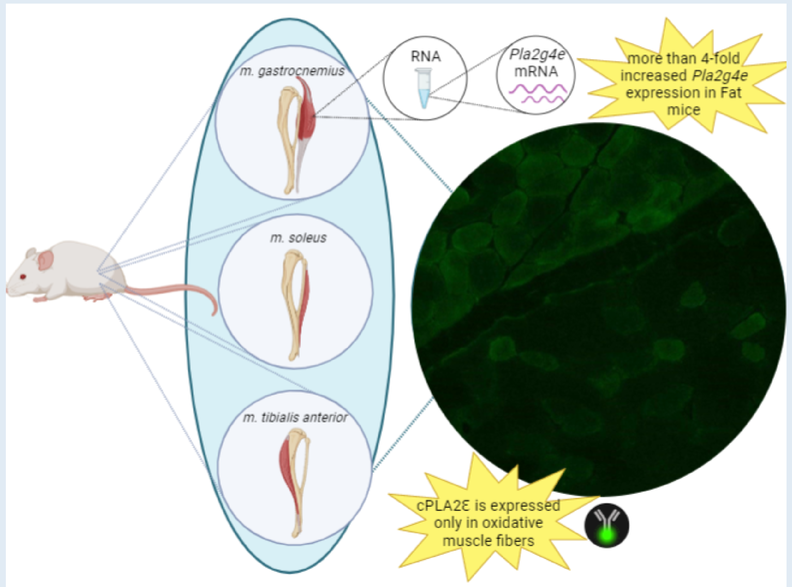
M. gastrocnemius, m. soleus, m. tibialis anterior:
- fluorescent immunostaining of cPLAε, the protein product of *Pla2g4e (*anti-Pla + ARF/ART)
- differential DAB-immunostaining of different muscle fibers (SC71/BFF3 + P260)
- muscle fiber type-related cPLAε expression analysis with Ellipse software

Bioinformatic analyses for determining CTCF and miRNA-binding sites and genome organization:
- TargetScan
- CTCFBSDB database

Obesity in Fat mice developed despite the same feed intake as Lean mice and regardless of the diet type.

Pla2g4e product, **cPLAε**, is synthesized only in **oxidative muscle fibers** (I and IIa).

Pla2g4e is **overexpressed** in skeletal muscles of **Fat mice** of both sexes. Polymorphisms in regulatory regions of Fat line may be affecting rate of transcription, miRNA silencing or RNA stability, leading to difference in protein expression. This results in **altered muscle aerobic respiration** that may contribute to obesity development.



CONCLUSION

These results point to the importance of genetic background in obesity development and suggest that *Pla2g4e* is one of the potential novel genetic factors highly linked to obesity that deserves further functional and mechanistic research.

RESULTS

BODY WEIGHT
Diet type affected body weight of mice. Mice on HFD were heavier than mice on LFD. The effect of genetic background on body weight was significant, as male and female mice of the Fat line were heavier than male and female mice of the Lean line (Figure 2), regardless of the diet type. Interestingly, their feed intake was the same.

***Pla2g4e* EXPRESSION IN SKELETAL MUSCLE**
The expression of *Pla2g4e* in *m. gastrocnemius* in Fat mice was several times higher than in Lean mice (Figure 3), regardless of the diet. A significant *Pla2g4e* fold change can be seen in both males and females.

cPLAε EXPRESSION PATTERN IN SKELETAL MUSCLE
cPLAε expression was determined immunohistochemically in type I (slow oxidative) and IIa (fast oxidative) fibers of skeletal muscles, which were present in small quantity in *m. gastrocnemius* and *tibialis anterior* and in greater quantity in *m. soleus* (Figure 4).

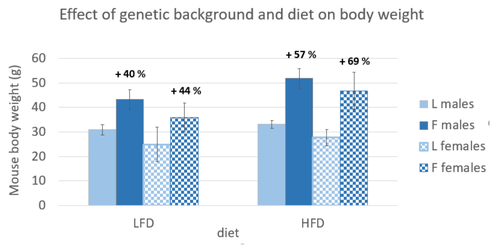


Figure 2: Mice of both sexes from the Fat line were heavier than mice from the Lean line in both diet treatments.

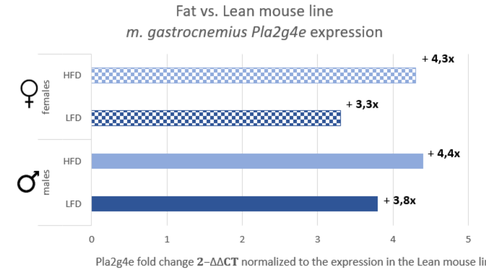


Figure 3: Expression of *Pla2g4e* in *m. gastrocnemius* represented as a fold change when comparing the Fat mouse line to the Lean mouse line.

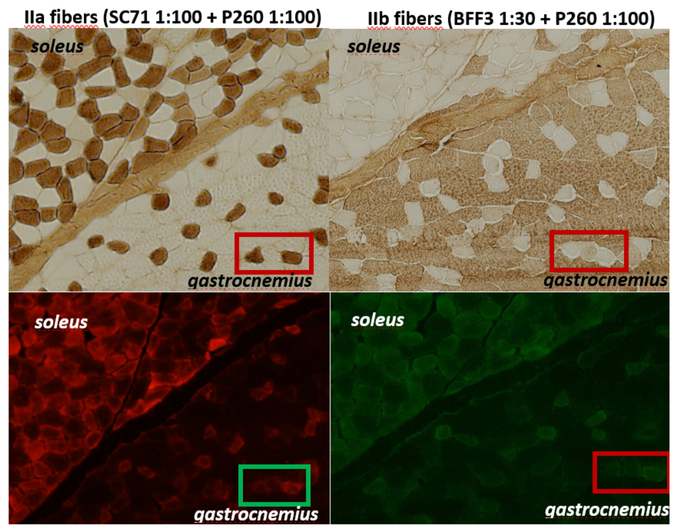


Figure 4: A zoomed section of interconnected *m. soleus* and *m. gastrocnemius* skeletal muscles of a control mouse after differential DAB-immunostaining for IIa and IIb muscle fibers (brown signal) and fluorescent immunostaining for cPLAε (ARF-red or ART-green signal).

BIOINFORMATIC ANALYSIS OF *Pla2g4e* REGION
Many miRNAs are predicted to be involved in post-transcriptional modifications of *Pla2g4e* . Genetic variants within predicted miRNA target sites in 3'UTR and CTCF binding motifs in the intronic region of *Pla2g4e* were found in Fat, but not in Lean mice --> possible protection in Fat mice from various silencing mechanisms resulting in an increased expression of *Pla2g4e* and therefore, in modified metabolism associated with obesity development.

Immunohistochemical results from Fat and Lean mice are still under analysis. Are there differences in cPLAε expression in skeletal muscle between the mouse lines or between sexes? Could differential cPLAε expression in muscle fibers be one of the causes for the development of metabolic syndrome and obesity in Fat line mice?