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Free communications in genetics

Investigating genes and enzymes related to intramuscular fat content in pig meat

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PROGETTO Ager AGROALIMENTARE E RICERCA FONDAZIONI IN RETE PER LA RICERCA AGROALIMENTARE

INNOVAGEN project, “Genomics in Italian heavy pig breeding”- MIUR

Introduction

Intramuscular fat (IMF) content is important for meat quality and one of the main goals of pork industry is to find an efficient strategy for balancing and/or enhancing IMF content.

The knowledge of genes involved in IMF deposition is still incomplete.

Aims

- Expression study of some genes related to IMF content

- Protein expression study of some enzymes related to IMF content

- Identification of possible co-expressions or interactions linking the considered genes and proteins.

Genes and proteins

	Role	Gene Expression	Protein Expression
Perilipin family: PLIN1; PLIN2; PLIN3; PLIN4; PLIN5	Coat intracellular lipid droplets	✓	
Adipose triglyceride lipase (ATGL)	Hydrolyses of triglycerides in adipose tissue	✓	
Hormone sensitive lipase (LIPE)	Hydrolyses stored triglycerides to free fatty acids	✓	✓
Lipoprotein Lipase (LPL)	Hydrolyses triglycerides to diglycerides	✓	✓
Monoglyceride Lipase (MGLL)	Converts monoacylglycerides to free fatty acids and glycerol	✓	
Stearoyl-CoA Desaturase ” 9 (SCD)	Introduces the first double bond into saturated fatty acyl-CoA substrates	✓	✓
Fatty Acid Synthase (FASN)	Catalyses the synthesis of palmitate from acetyl-CoA and malonyl-CoA	✓	✓

Sampling

The most extreme individuals for IMF content (IMF < 1.50% or IMF > 3.50%) were chosen among a population of 950 Italian Large White pigs, avoiding relatives.

	GENE EXPRESSION			PROTEIN QUANTITATION		
	LOW IMF samples (IMF<1.50%)	**HIGH IMF samples (IMF>3.50%)**	**N° Total**	**LOW IMF samples (IMF<1.50%)**	**HIGH IMF samples (IMF>3.50%)**	**N° Total**
N° Castrated males	11	10	21	21	28	49
N° Sows	14	12	26	56	50	106
N° Total	25	22	47	77	78	155
IMF mean (%) ± st. dev.	0.74 ± 0.09	5.72 ± 1.13		0.84 ± 0.13	4.66 ± 1.03	

Materials and Methods

Semimembranosus muscle samples

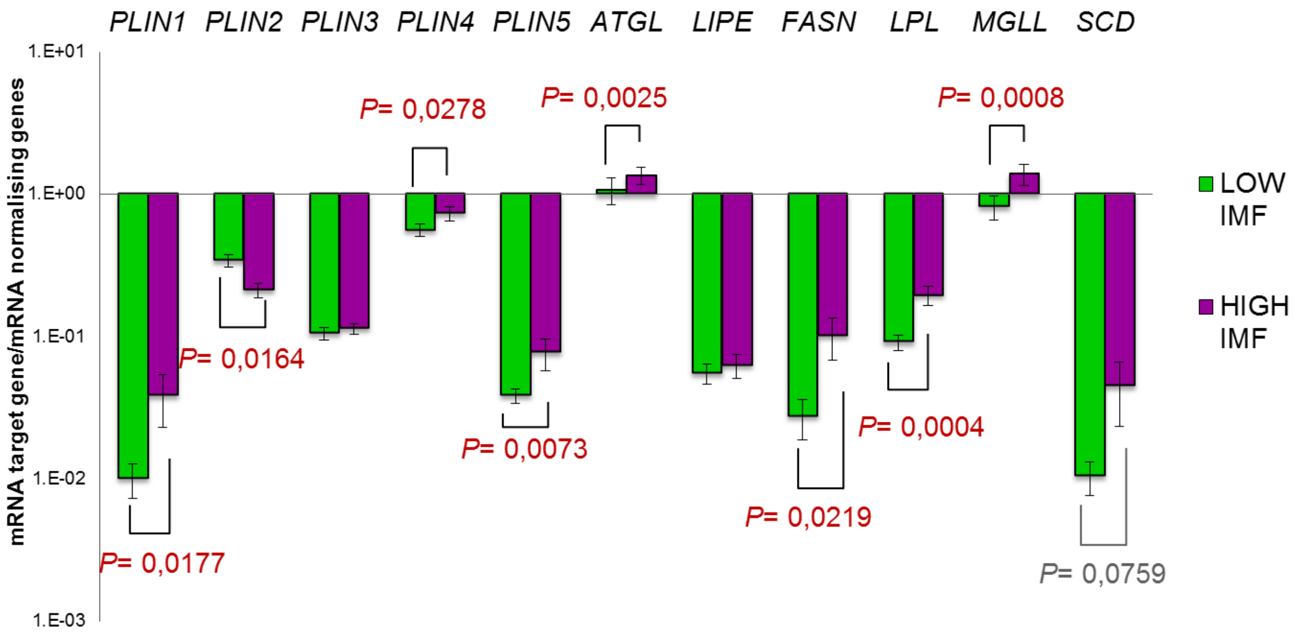
For gene expression study:

- Total RNA extraction and retrotranscription
- qRT-PCR on Rotor Gene 6000 through standard curves with serial dilutions
- Normalising genes: *B2M*, *YWHAZ*, *HPRT1*
- Student’s t test to compare groups
- Considered genes co-expressions through partial correlation analysis

For protein quantitation:

- Total protein extraction
- Total protein quantitation
- The specific enzyme quantitation through ELISA kit
- Wilcoxon test to compare the data
- Correlation analysis

Gene Expression Results



PLIN1*, *PLIN4*, *PLIN5*, *ATGL*, *FASN*, *LPL*, *MGLL

SCD (trend in significance)

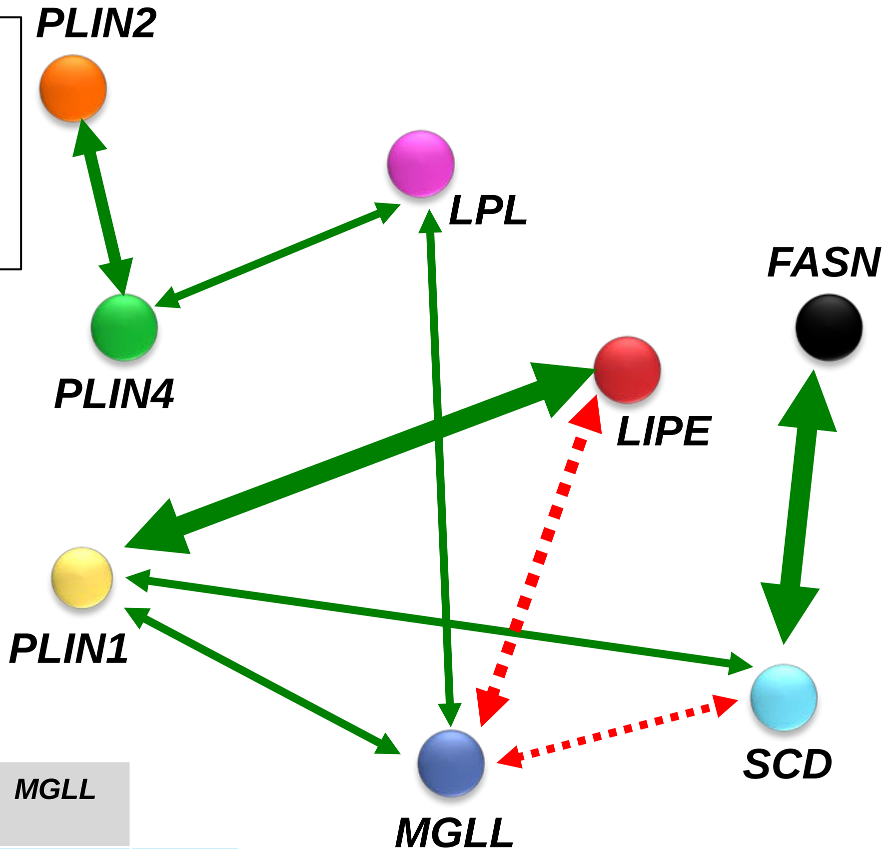
} ↑ in **HIGH IMF samples**

PLIN2 ↑ in **LOW IMF samples**

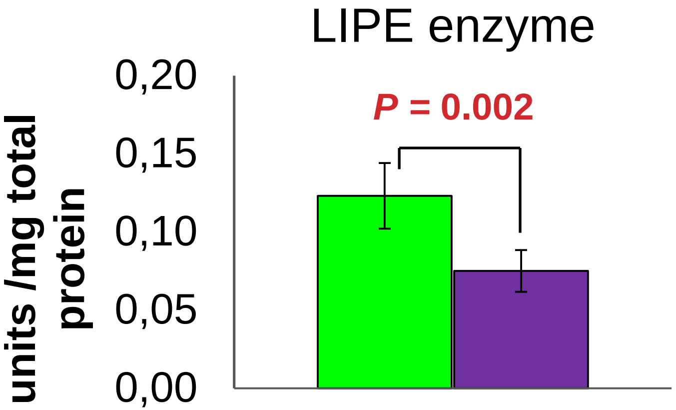
Genes co-expression

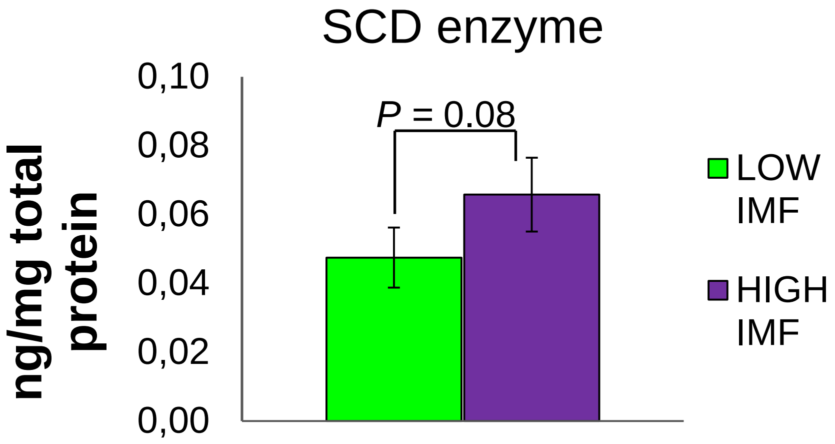
Transcription level correlations highlighted a complex genes co-expression pathway linking in particular *LIPE* with *PLIN1*, and *FASN* with *SCD*.

PLIN1							
-	***PLIN2***						
-	r = 0.51 P < .001	***PLIN4***					
r = 0.77 P < .0001	-	-	***LIPE***				
-	-	-	-	***FASN***			
-	-	r = 0.38 P < .05	-	-	***LPL***		
r = 0.37 P < .05	-	-	r = -0.51 P < .001	-	r = 0.37 P < .05	***MGLL***	
r = 0.40 P < .05	-	-	-	r = 0.79 P < .0001	-	r = -0.37 P < .05	***SCD***



Protein Quantitation Results





LIPE is the only protein among the tested enzymes showing differences in its amount between LOW IMF samples and HIGH IMF samples, with higher amounts in LOW IMF individuals.

SCD enzyme was more expressed in HIGH IMF pigs, presenting a trend towards significance.

Protein level correlation results

The level of **FASN** resulted highly correlated with **LIPE** enzyme only in **female individuals**

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Sex dependent regulation pathway?

	LOW IMF group	HIGH IMF group	Sows	Castrated males
FASN enzyme (ng/mg Prot) with LIPE enzyme (u/mg Prot)	r = 0.741 P < .0001	r = 0.833 P < .0001	r = 0.911 P < .0001	r = 0.263 P = 0.096

Conclusions

- All *PLIN* genes, except for *PLIN3,* showed transcription levels associated with IMF deposition.

- *ATGL*, *FASN*, *LPL*, *MGLL* genes showed higher mRNA levels in pigs with HIGH IMF deposition compared to LOW IMF ones.

- The protein quantitation results suggest a sex-dependent pathway regulating FASN and LIPE protein expression levels.

- This study indicates that IMF deposition is related to a complex gene co-expression pathway and that LIPE protein quantitation may be a useful parameter indicating pigs predisposition towards IMF deposition.

THANKS FOR YOUR ATTENTION!

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GLM MODEL:

$$y = \mu + group + sex + batch + e$$

where:

y is the gene or protein expression level;

µ is the average expression level;

group stands for the two groups, LOW IMF Group coded as 1 and HIGH IMF Group coded as 2;

sex is the sex of the animal;

batch, the slaughtering batch of the animal

e is the error.

	FASN protein	LIPE protein	LPL protein	SCD protein	FASN gene expr.	LIPE gene expr.	LPL gene expr.	SCD gene expr.
Group	NS	0.021	NS	0.050	0.028	NS	0.011	0.051
Sex	NS	NS	NS	NS	NS	NS	NS	NS
Batch	0.048	NS	NS	NS	NS	NS	NS	NS