Co-association and gene network analysis for pig intramuscular fatty acid composition

from GWAS to Gene Network

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Why Fatty Acids?

- Moderate to high heritabilities (0.15 -0.55)

- Major energy source and important constituents of membrane lipids.

- Relevant role as cellular signalling molecules in various metabolic pathways.

- Technological, nutritional and organoleptic transformation of pork meat quality is highly dependent of lipid content and fatty acids composition.

- Positive impact in human health (PUFA, mainly ω-3)

Proc Natl Acad Sci U S A. 2010 Jul 19. [Epub ahead of print]

Association weight matrix for the genetic dissection of puberty in beef cattle.

Fortes MR, Reverter A, Zhang Y, Collis E, Nagaraj SH, Jonsson NN, Prayaga KC, Barris W, Hawken RJ.

PNAS
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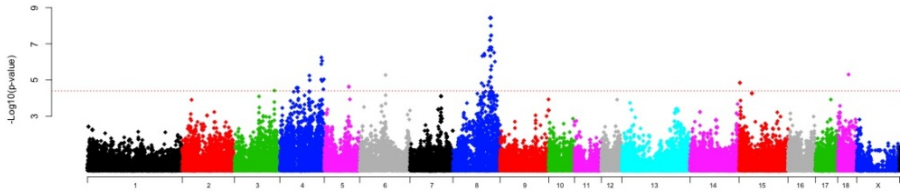
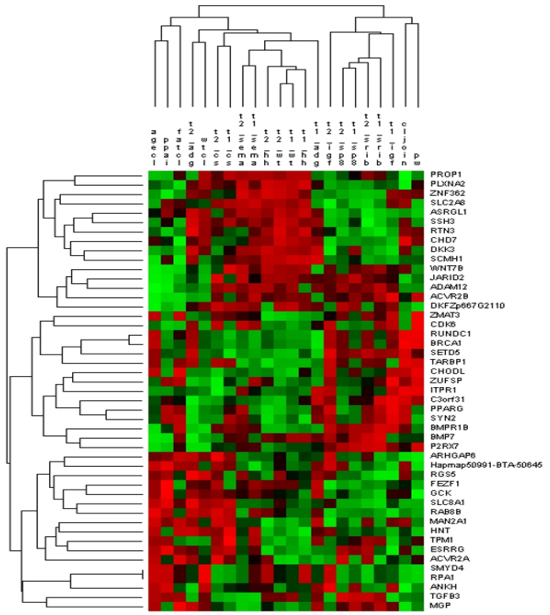
AWM

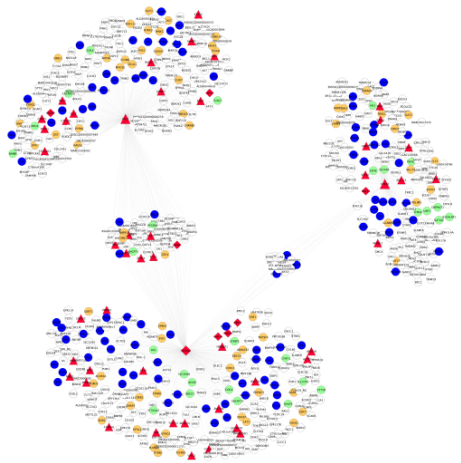
1. Combine the knowledge from GWAS
2. With the power of Transcription Regulators
3. In a Network Theory framework

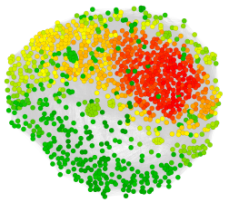
GWAS (P-value & â)



AWM







PCIT + Cytoscape

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Building the Association Weight Matrix

Selection of SNPs

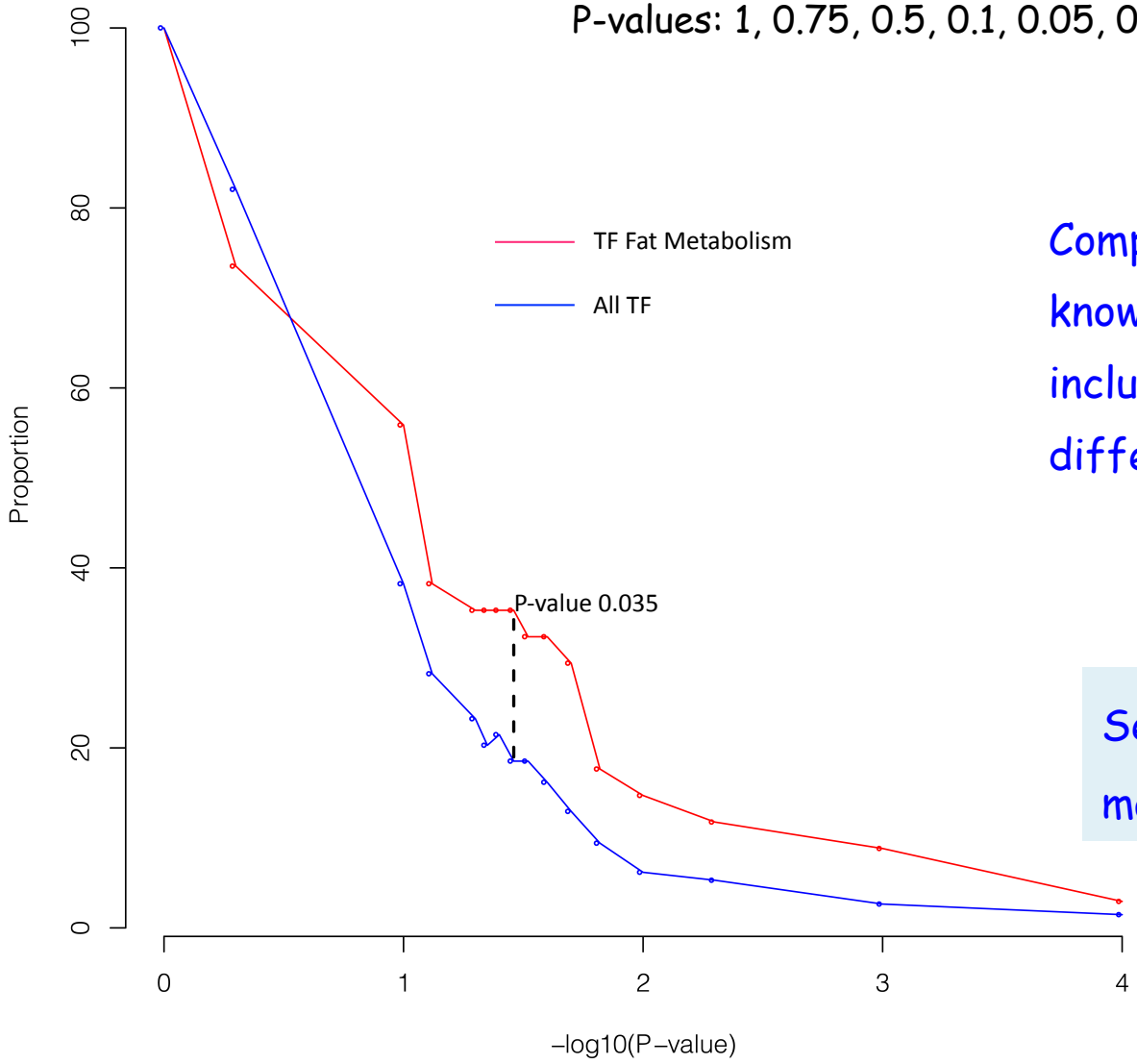
1. Select SNPs that "minimally" affect Key-Phenotype (ie. $P < 5\%$)
2. On average, how many of the remaining traits are affected by these Key-associated SNPs? ("μ")
3. Select SNPs with $P < 5\%$ in $\geq$ "μ " traits
4. Identify the nearest gene (cis-logic)
5. Select the gene-SNP with largest average effect across all traits

Alternative approach to select p-value thresholds

Exploring the sensitivity of p-value thresholds

to identify TF involved in fat metabolism

P-values: 1, 0.75, 0.5, 0.1, 0.05, 0.045 ….10^{-4}



Compare the proportion of the well known fat-related TF against all TF included in the data set, at different P-values.

Select the P-value cut-off that maximizes this difference.

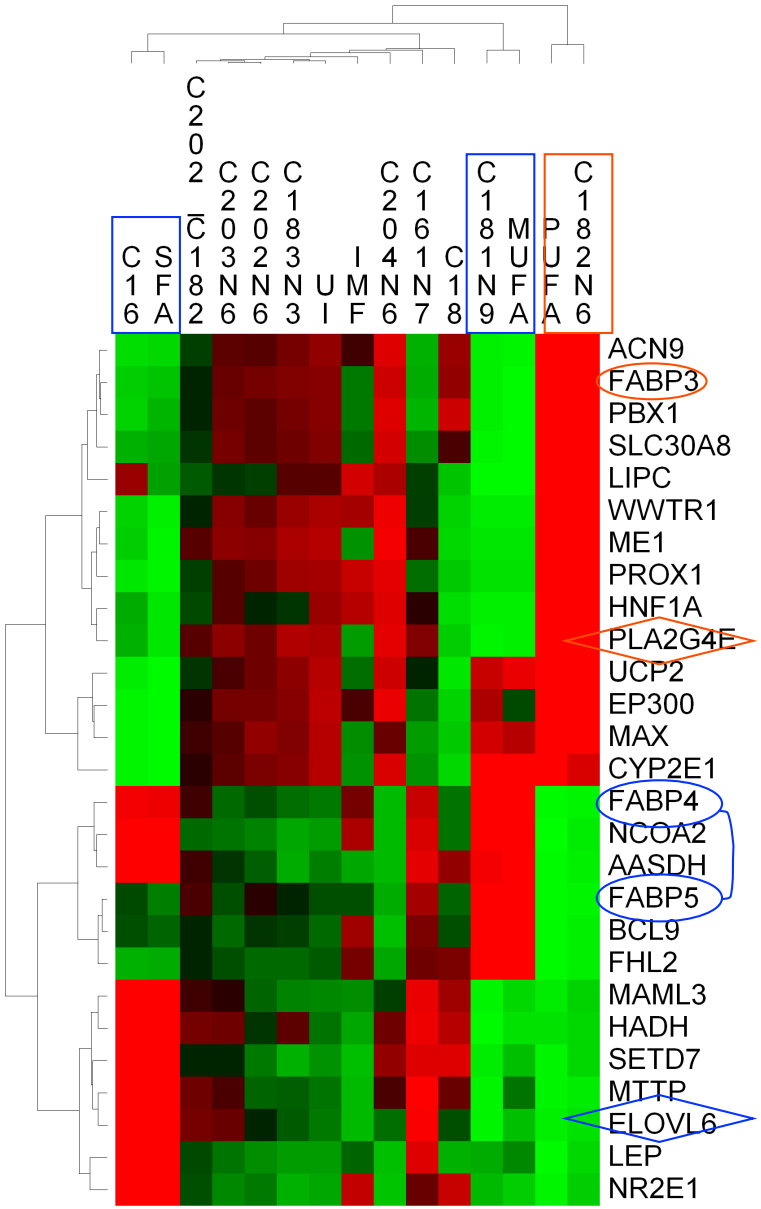
The Association Weight Matrix

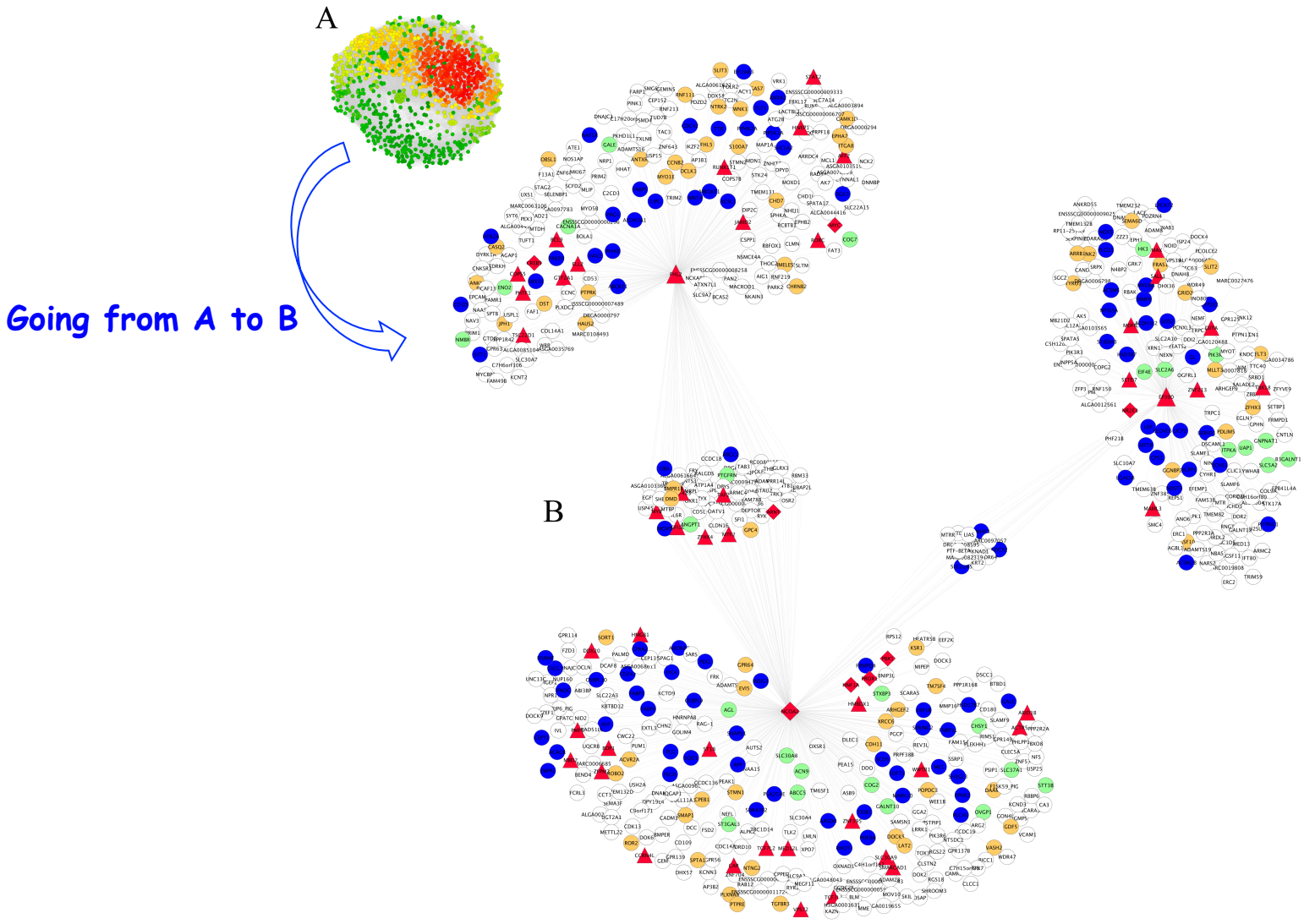
IMF Fatty Acid Composition

- Select SNPs that "minimally" affect C16:1 (n-7)
- On average, how many of the remaining 14 traits are affected by these C16:1-associated SNPs?
- Select SNPs with *P* <3.5% in ≥3 traits
- Identify the nearest gene (cis-logic)
- For genes represented by more than 1 SNP, select the SNP with largest effect average across all 15 traits

- 1,096 Genes as rows (via neighbouring SNP)
- 15 Traits as columns
- Each cell *{i,j}* value is the normalized (z-scores) additive effect of i^{th}-SNP on the j^{th}-trait

Hierarchical tree cluster





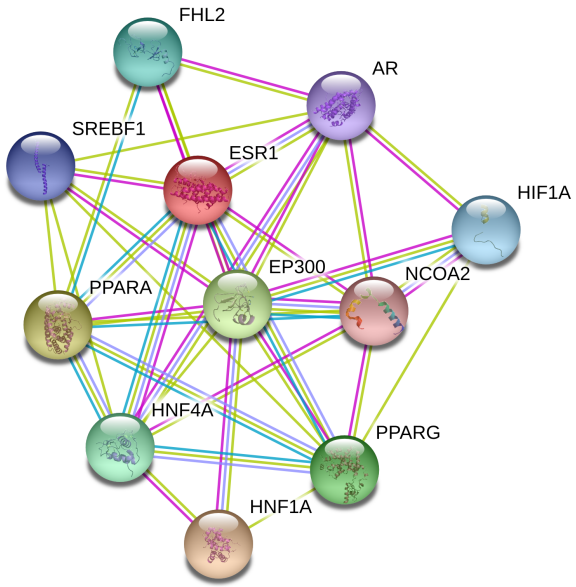
Top Trio: minimal set of TF that expanded the majority of the network topology

(*NCOA2, FHL2, EP300*)

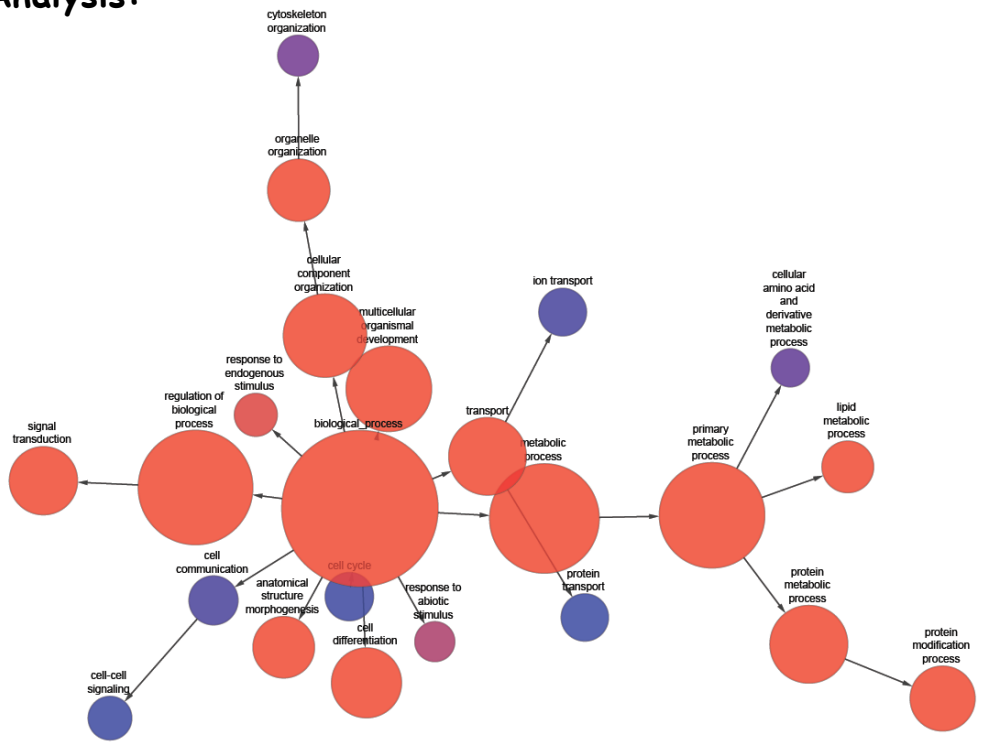
TFBS for key regulators of the lipid metabolism are present in the promoter region of the three top TF

TF	TFBS for
EP300	**HNF1A, PPARG, PPAR-α, SREBP-1a, SREBP-1b, SREBP-1c, SP1, ER- α, GR-α**
FHL2	**HNF4- α1, HNF4- α2, HNF1A, PPARG, PPAR-α,** CREB, p300
NCOA2	**HNF4- α1, ER- α, GR-α, ARNT, HNF4- α2, HNF1A, PPARG, PPAR-α,**p300

Experimental data confirmed that protein-protein interaction exist among the three TF and those master regulators of lipid and carbohydrate metabolism (String database: http://string-db.org/)

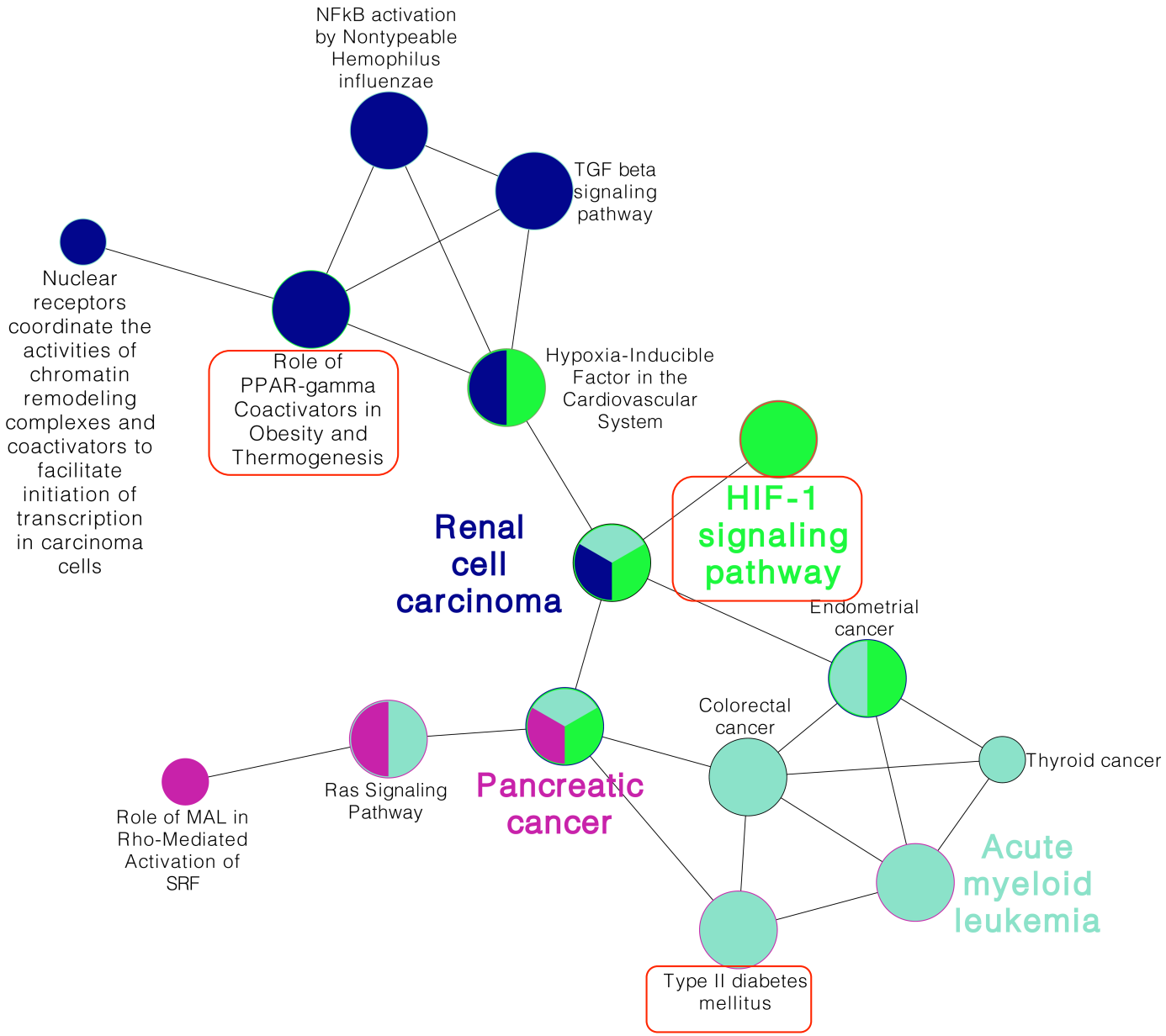


GO Enrichment Analysis:



Description	Total	P-value	FDR
cellular component organization	206	4.74×10^{-10}	1.82×10^{-8}
cell differentiation	138	1.12×10^{-7}	2.57×10^{-6}
anatomical structure morphogenesis	102	3.49×10^{-6}	3.88×10^{-5}
cytoskeleton	107	1.17×10^{-4}	5.97×10^{-4}
lipid metabolic process	67	5.71×10^{-4}	2.05×10^{-3}
cytoskeleton organization	37	$6.22E \times 10^{-3}$	1.79×10^{-2}
lipid binding	33	1.34×10^{-2}	3.36×10^{-2}
cell-cell signaling	44	1.59×10^{-2}	3.80×10^{-2}

Pathway Enrichment Analysis:



39 of the AWM-predicted target genes have been recently reported in two large-scale meta-analysis for plasma lipids in humans

nature International weekly journal of science

Biological, clinical and population relevance of 95 loci for blood lipids

AJHG The American Journal of Human Genetics

ARTICLE

Large-Scale Gene-Centric Meta-analysis across 32 Studies Identifies Multiple Lipid Loci

Literature evidence:

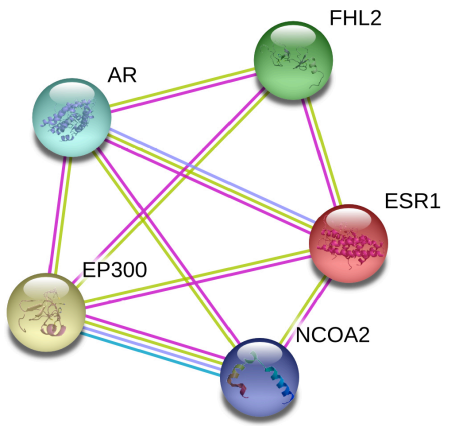
NCOA2 (Energy homeostasis & AR co-regulator)

EP300 (Transcription regulation via chromatin remodelling, required for adipocyte differentiation, co-activator of *AR, ER, PPAR-γ, HNF4, ARNT* and *LXR*)

FHL2 (co-activator of *AR, ER* and *PPAR-α*)

Functional co-operation:

- *NCOA2* and *EP300* belong to AR and ER pathways
- *NCOA2* and *FHL2* are AR co-regulators
- Cooperation activation of AR-mediated transcription among *EP300*, *FHL2* and *β-catenin*



String database: http://string-db.org/

Conclusions

-AWM points to new candidate genes, TF and gene interactions via exploring SNP co-associations across multiples traits beyond the one-dimensional approach for identifying genes affecting single traits.

-Network predictions, supported by bibliography and co-expression analysis suggest a co-operative role for the three TF in the transcriptional regulation of IMF, FA composition in pigs.

-Our study provides additional evidences to support the usefulness of pig as an animal model for the study of metabolic diseases in humans.

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