



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA



Animal and Food Genomics Group

Integrating genomic information in metabolomic networks to dissect molecular phenotypes in pigs

Samuele Bovo

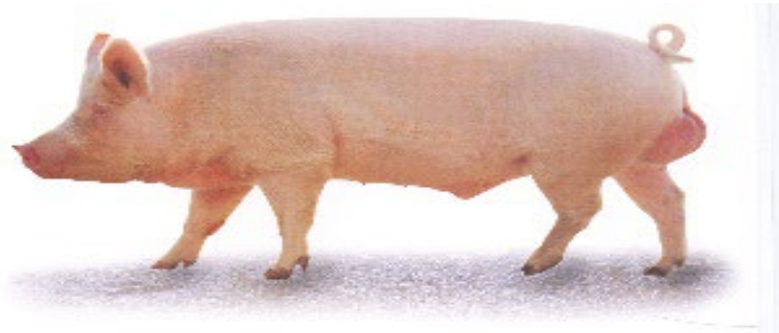
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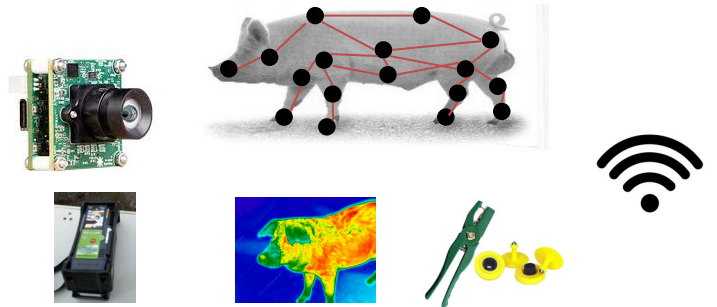


The animal phenome

Acquisition of high-dimensional phenotypic data on an organism-wide scale

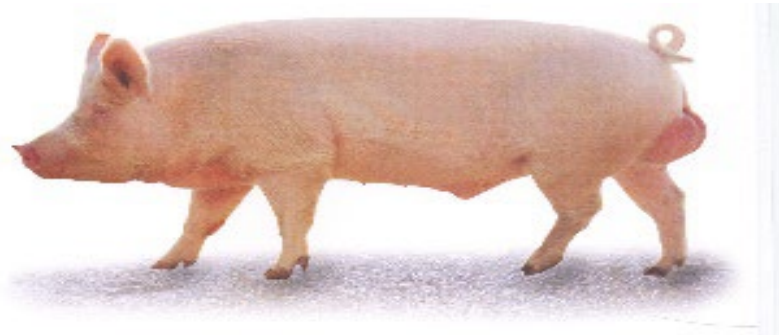


Traditional phenotypes
Behavioral traits
Gas emissions
Feed efficiency
...

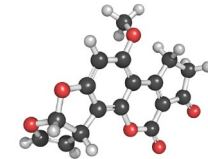
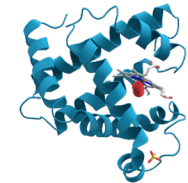


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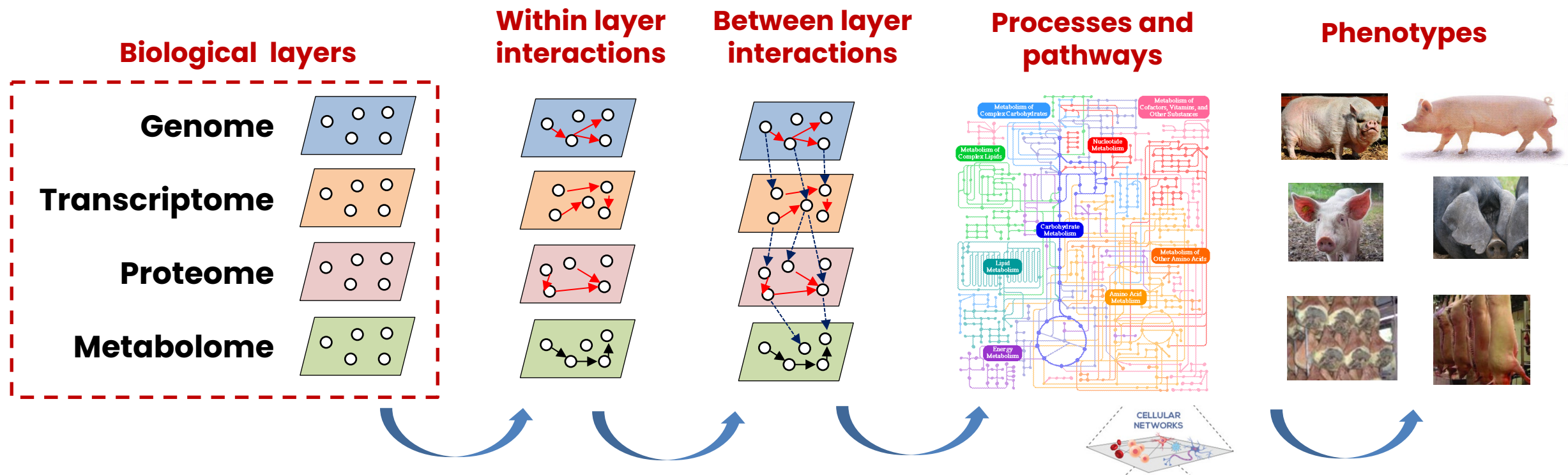


Traditional phenotypes
Behavioral traits
Gas emissions
Feed efficiency
Molecular Phenotypes



Cell counts, Metabolites, Proteins, Expression, ...

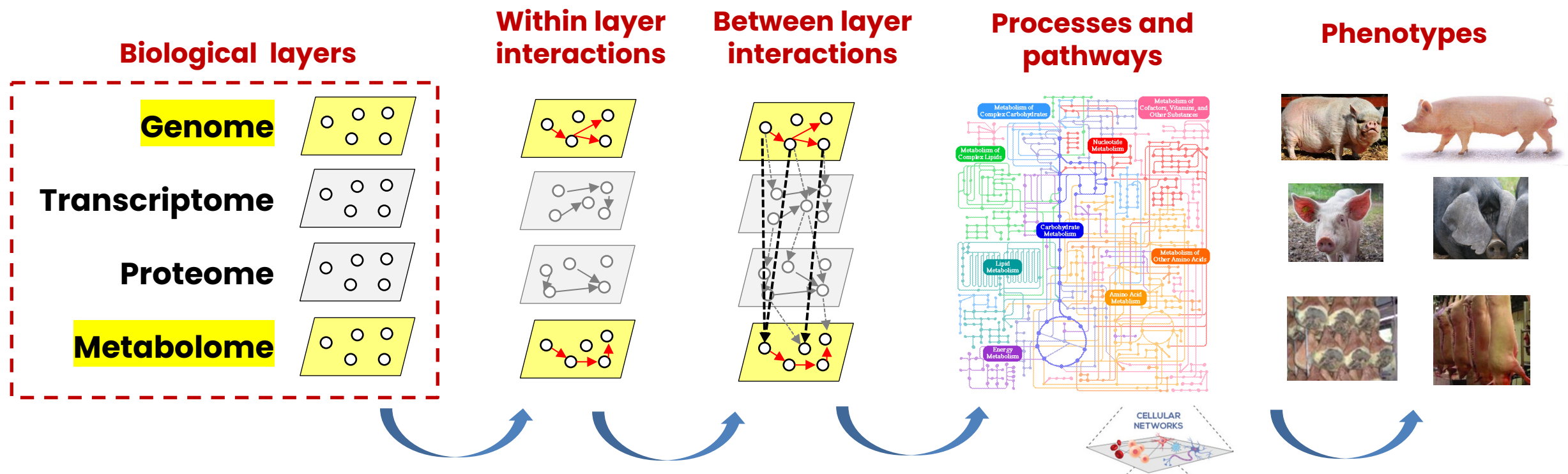
Biological complexity and the animal phenotype



Intermediate phenotypes == simpler phenotypes
→ They act as a proxy for the final phenotype

Complexity
Emergent properties

Biological complexity and the animal phenotype



Exploring and gaining knowledge on the interplay between these two layers

Exploring and gaining knowledge on the interplay between these two layers.

How?

- 1. Metabolites → Networks of metabolites**
- 2. Metabolites + DNA markers → Discovery of DNA markers affecting metabolite levels**

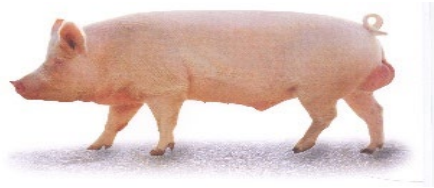


- 3. Data integration**

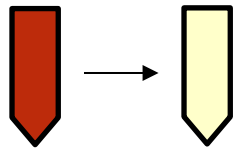


Experimental materials

Animals



~700 Italian Large White



Blood

Plasma

SIB test (ANAS)

1/3 castrated males, 2/3 gilts

155 ± 5 kg live weight

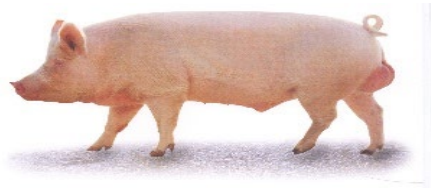
Similarly raised

17 sampling days

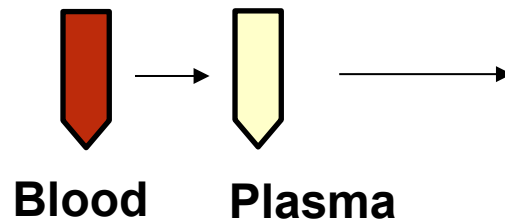


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Targeted Metabolomics (~200 metabolites)

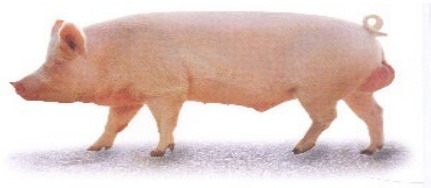
Metabolite classes	No.	Biological relevance (selected)
Acylcarnitines	40	Energy metabolism fatty acid transport
Amino acids	21	Amino acid metabolism, neurotransmitter metabolism
Biogenic amines	19	Neurological disorders DNA stability, oxidative stress
Hexoses	1	Carbohydrate metabolism
Glycerophospholipids	90	
- lysoPhosphatidylcholine acyl – lysoPC a Cx:x	14	Degradation of phospholipids fatty acid profile
- Phosphatidylcholine diacyl – PC aa Cx:x	38	Dyslipidemia, Membrane composition and damage
- Phosphatidylcholine acyl-alkyl – PC ae Cx:x	38	
Sphingolipids	15	Signalling cascades membrane damage

Absolute quantification of metabolites (HPLC + MS/MS)



Experimental materials

Animals



Genotyping



~60K SNPs

~700 Italian Large White



Blood



Plasma

SIB test (ANAS)
1/3 castrated males, 2/3 gilts
155 ± 5 kg live weight
Similarly raised
17 sampling days



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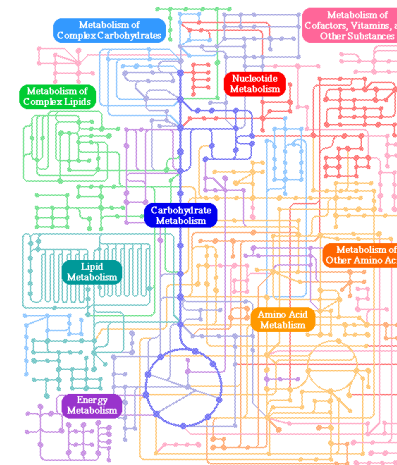
Absolute quantification of metabolites (HPLC + MS/MS)



1. Reconstructing the the pig metabolism



Network generation



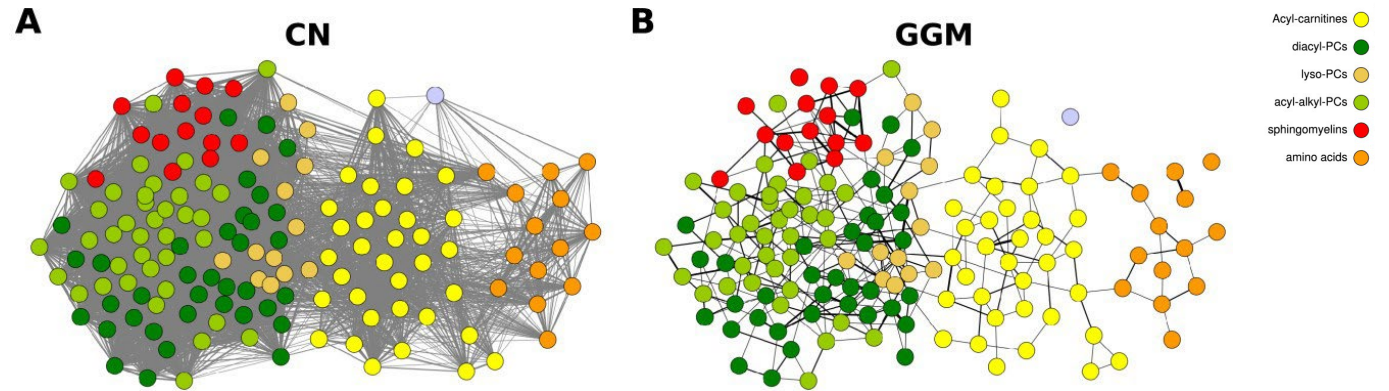
Network generation

Two approaches:

1. Correlation Network (CN)
2. Gaussian Graphical Model (GGM)

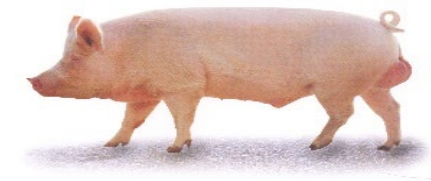
Differences between :

- CN: simple correlation (r)
- GGM: partial correlation (PCC)



PCC = pairwise correlation between two variables conditioned against the correlations with all other variables.

GGM: If the variables are still correlated, the correlation is directly determined by the association of X and Y and not mediated by the other variables.



Data processing and GGM generation in pigs

186 metabolites

Raw profiles



CV% metabolites
Missing values
Outlier samples

HQ profiles



Linear models
→ removal of
confounding effects

Residuals

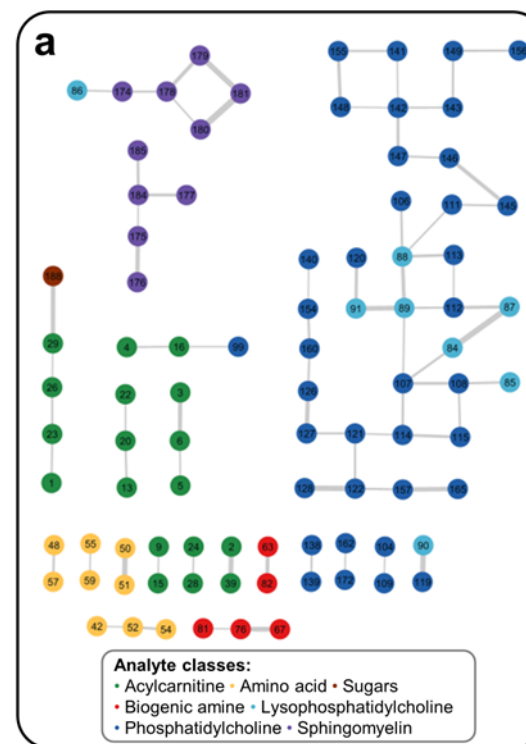


164 metabolites



CN

GGM



PCC > 0.3

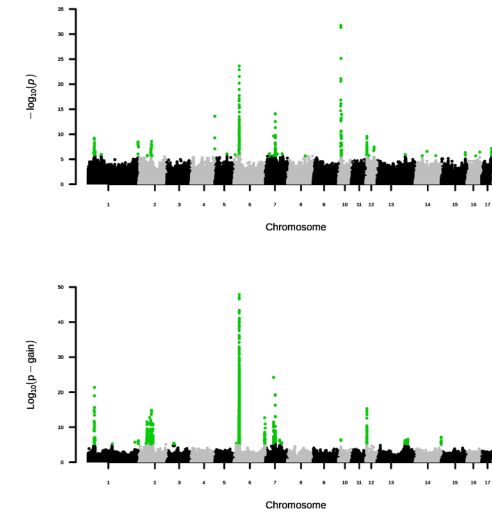
GGM features:

- 88 nodes x 73 edges
- 71 singletons
- Clusters with metabolites of the same biological class
- Few inter-class connections

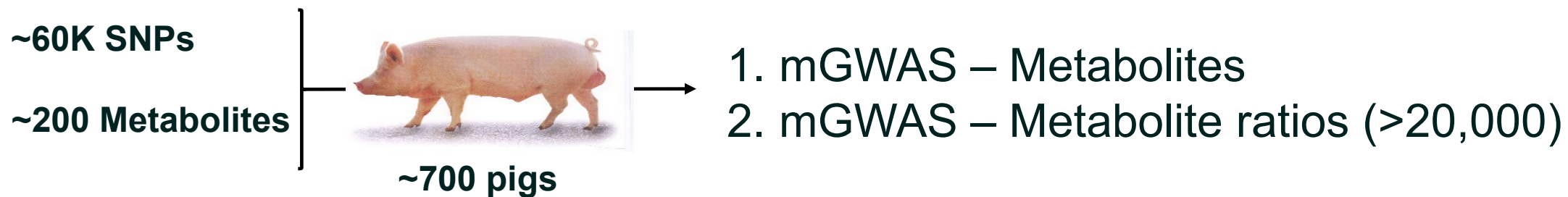
2. Discovery of DNA markers affecting metabolite levels



GWAS analyses

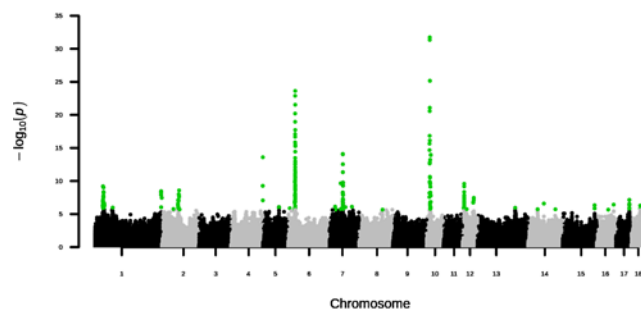


Metabolomics and Genomics in pigs

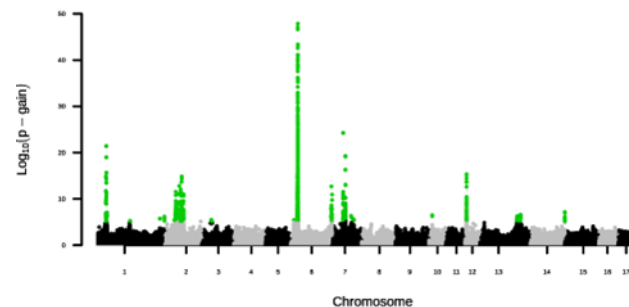


33mQTL
56 metabolites
594 ratios

mGWAS – Metabolites



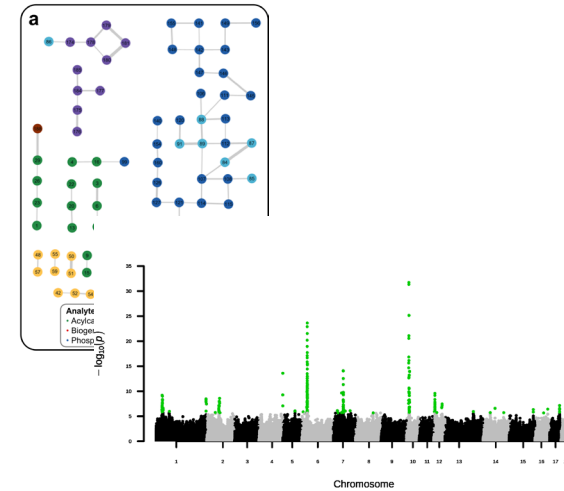
mGWAS – Ratios



3. Data integration



**Network
+
GWAS results**

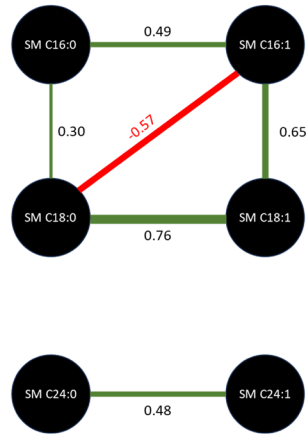


Merging of information: a qualitative approach

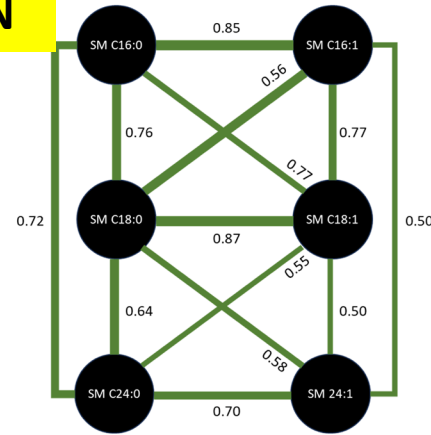
The sphingomyelin pathway



GGM



CN



Differences between GGM and CN

- Less connected
- **Negative correlations**

- Occur whenever regular negative corr. are involved;
- Due to the mathematical properties of PCC.

Krumsiek et al. *BMC Systems Biology* 2011, 5:21
<http://www.biomedcentral.com/1752-0509/5/21>

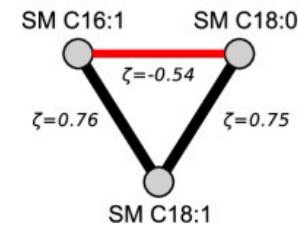


RESEARCH ARTICLE

Open Access

Gaussian graphical modeling reconstructs pathway reactions from high-throughput metabolomics data

Jan Krumsiek¹, Karsten Suhre^{1,2}, Thomas Illig³, Jerzy Adamski⁴, Fabian J Theis^{1,5*}



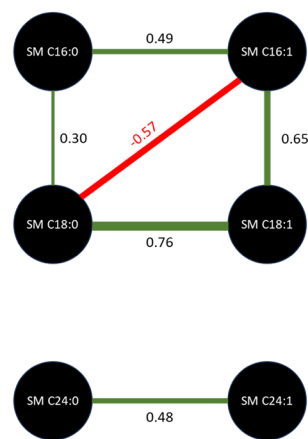
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Merging of information: a qualitative approach

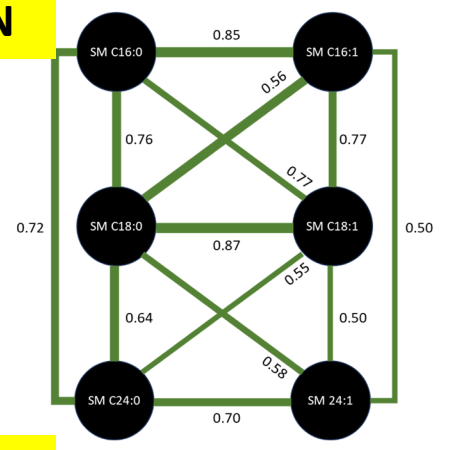
The sphingomyelin pathway



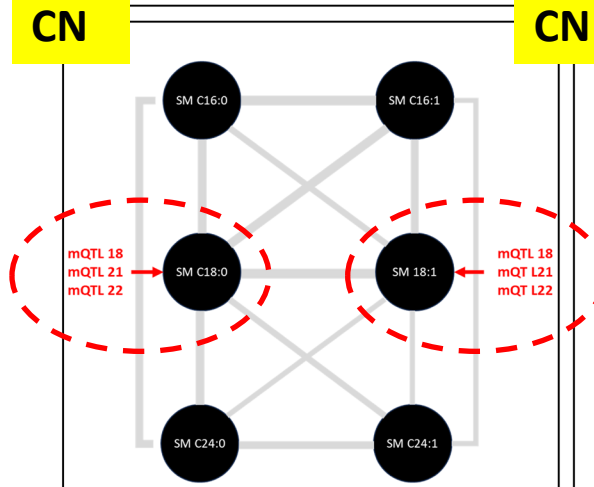
GGM



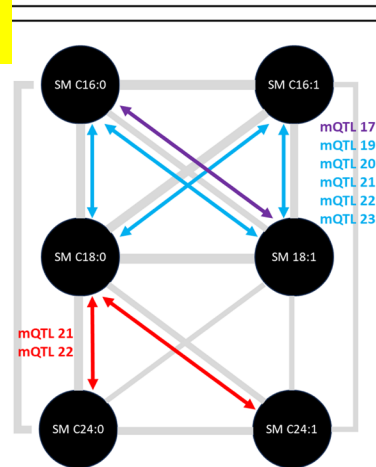
CN



CN



CN



mQTLs

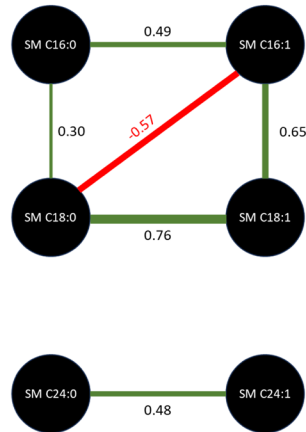
- affecting specific metabolites

Merging of information: a qualitative approach

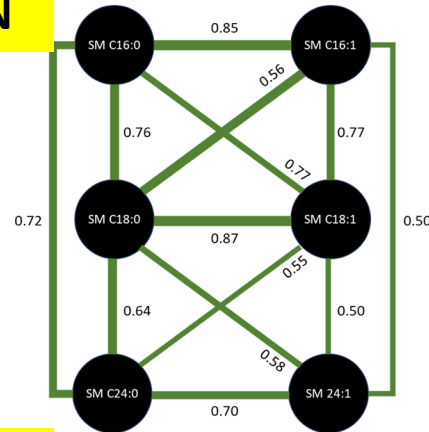
The sphingomyelin pathways



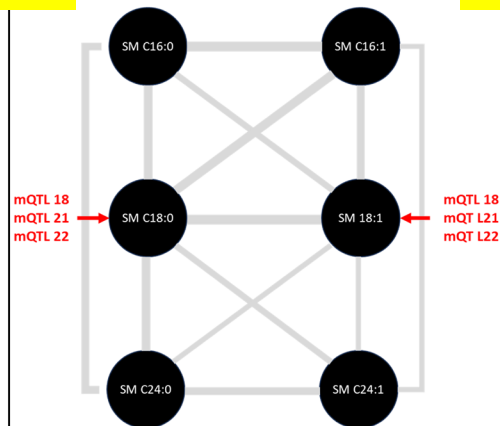
GGM



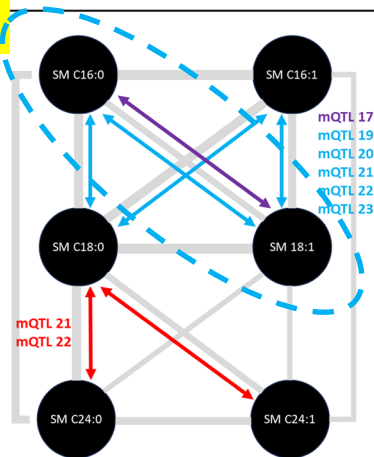
CN



CN



CN



mQTLs

- affecting specific metabolites
- affecting specific metabolites and some of their ratios

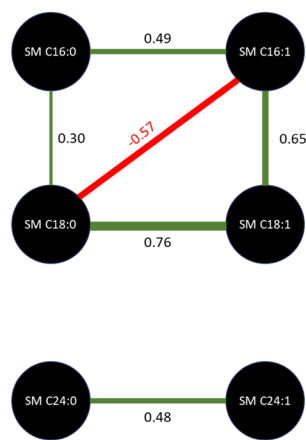


Merging of information: a qualitative approach

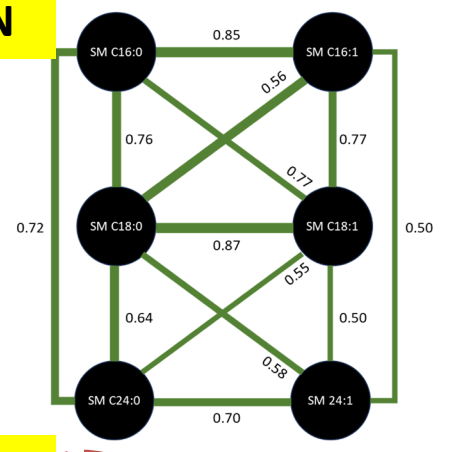
The sphingomyelin pathway



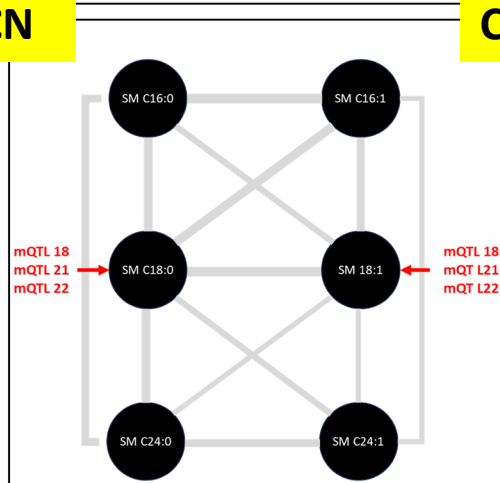
GGM



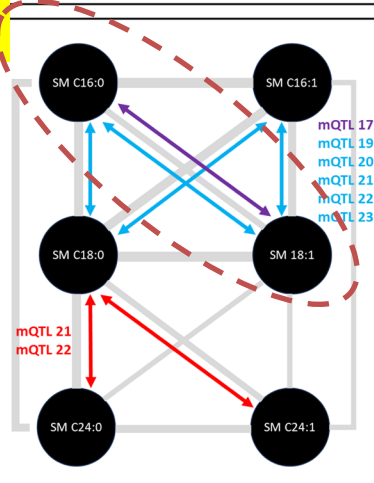
CN



CN



CN



mQTLs

- affecting specific metabolites
- affecting specific metabolites and some of their ratios
- Specific for the ratio(s) (but not for the single metabolite)



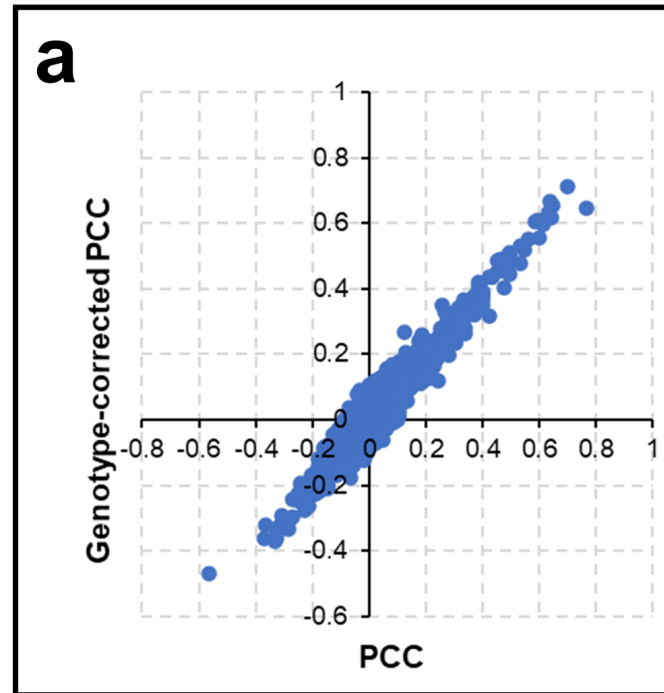
Merging of information: a quantitative approach

Inclusion of DNA information:

1. **Metabolites regressed over their associated DNA markers.**
2. Residuals are obtained.
3. Computation of the CN and GGM with the residuals.



GGM



Few differences

Krumsiek et al. *BMC Systems Biology* 2011, 9:21
<http://www.biomedcentral.com/1752-0509/5/21>



RESEARCH ARTICLE

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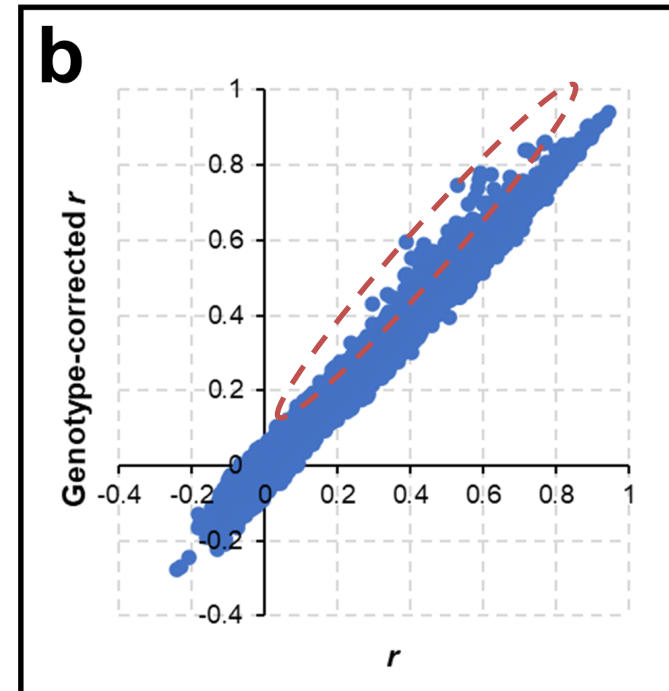
The genetic effect is neglectable indicating that GGMs capture intrinsic biochemical properties of the system.



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What about the effect over the CN?

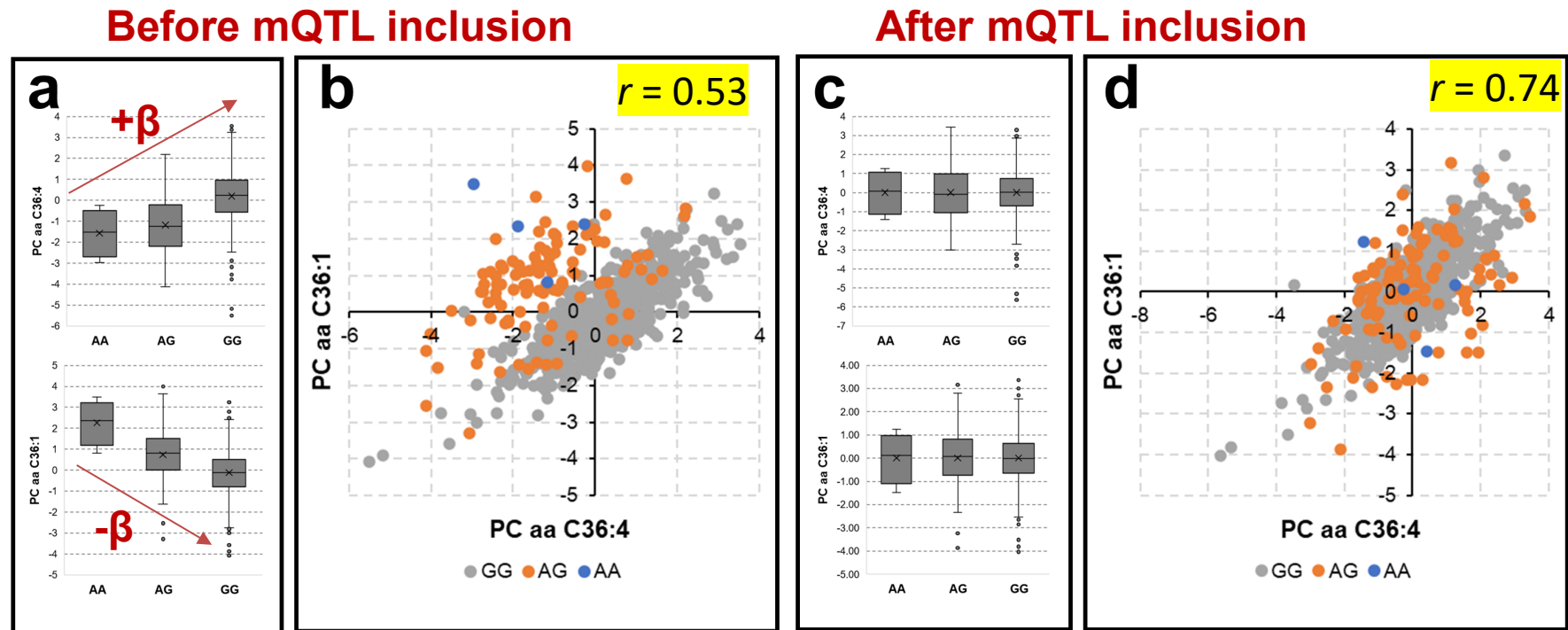
- The impact of the genetic information was more pronounced;
- A total of 25 correlations had $\Delta r > 0.2$;
- Enrichment of some metabolites
 - PC aa C36:4 (in 8 correlation)
 - PC aa C36:1 (in 6 correlations)



What about the effect over the CN?

- The pair PC aa C36:4–PC aa C36:1 → from $r = 0.53$ to $r = 0.74$
- Both metabolites have been associated with the same QTL

Different effect
(opposite β)



Conclusions

1. Relationships between metabolites can not be disclosed with metabolomics solely;
2. GWAS of metabolite ratios complement results of network approach → More detailed picture of the metabolism;
3. Methodology: the effect of genetic information over the network
 - can be neglectable considering PCC (GGM);
 - can be severe if working with simple correlations (CN).



Animal and Food Genomics Group



Luca Fontanesi
Full Professor



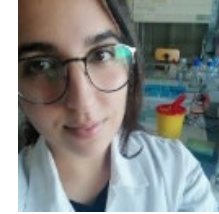
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Associate Professor



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Junior Assistant Professor
Molecular genetics



Valeria Taurisano
Post-doc
Molecular genetics



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Data analysis



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Associate Professor



Jacopo Vegni
Post-Doc
Data analysis



Acknowledgments



Thank you !
Questions ?



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