The added value of molecular phenotypes: towards the identification of animal welfare proxies

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The added value of molecular phenotypes: towards the identification of animal welfare proxies…..

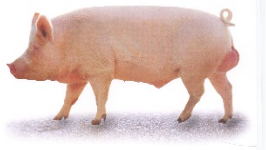
for breeding and selection purposes

Animal welfare
is a multi-dimensional concept:

- **From a pure biological approach:**

- ✓ Behaviour

    - ➢ Physiology
    - ➢ Immunology
    - ➢ Biochemistry
    - ➢ …



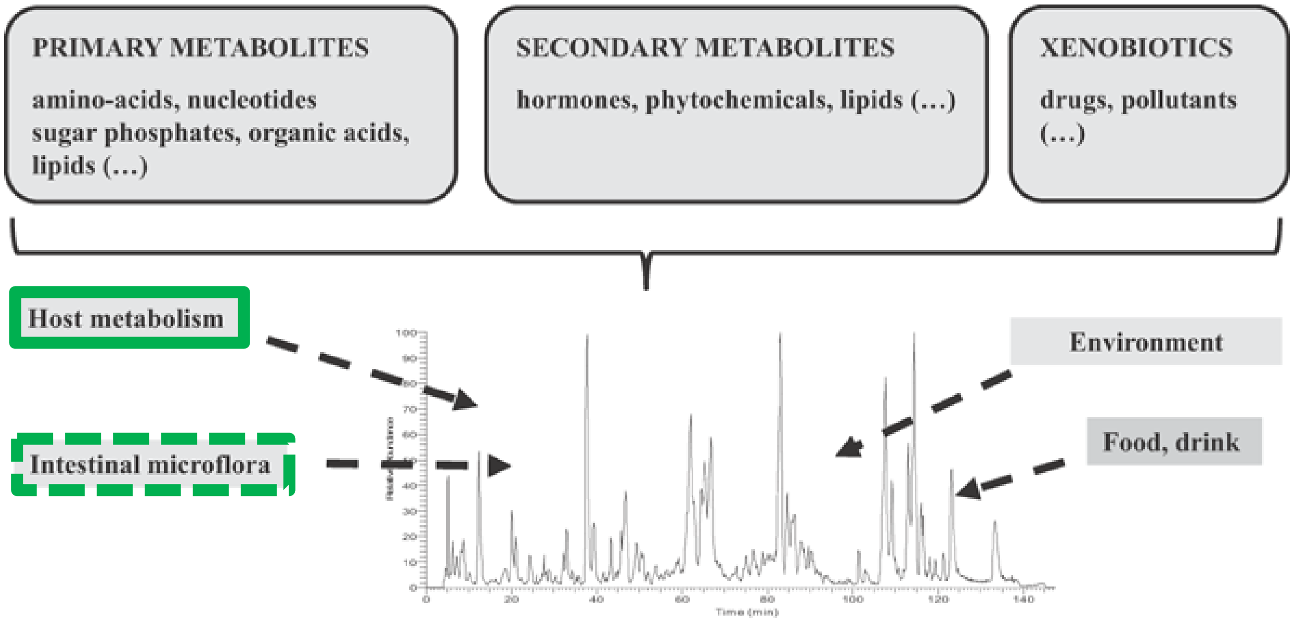
Our omics study in pigs

It combines:

- Metabolomics
- Genomics

- Hypothesis free approach
- Hypothesis generating approach

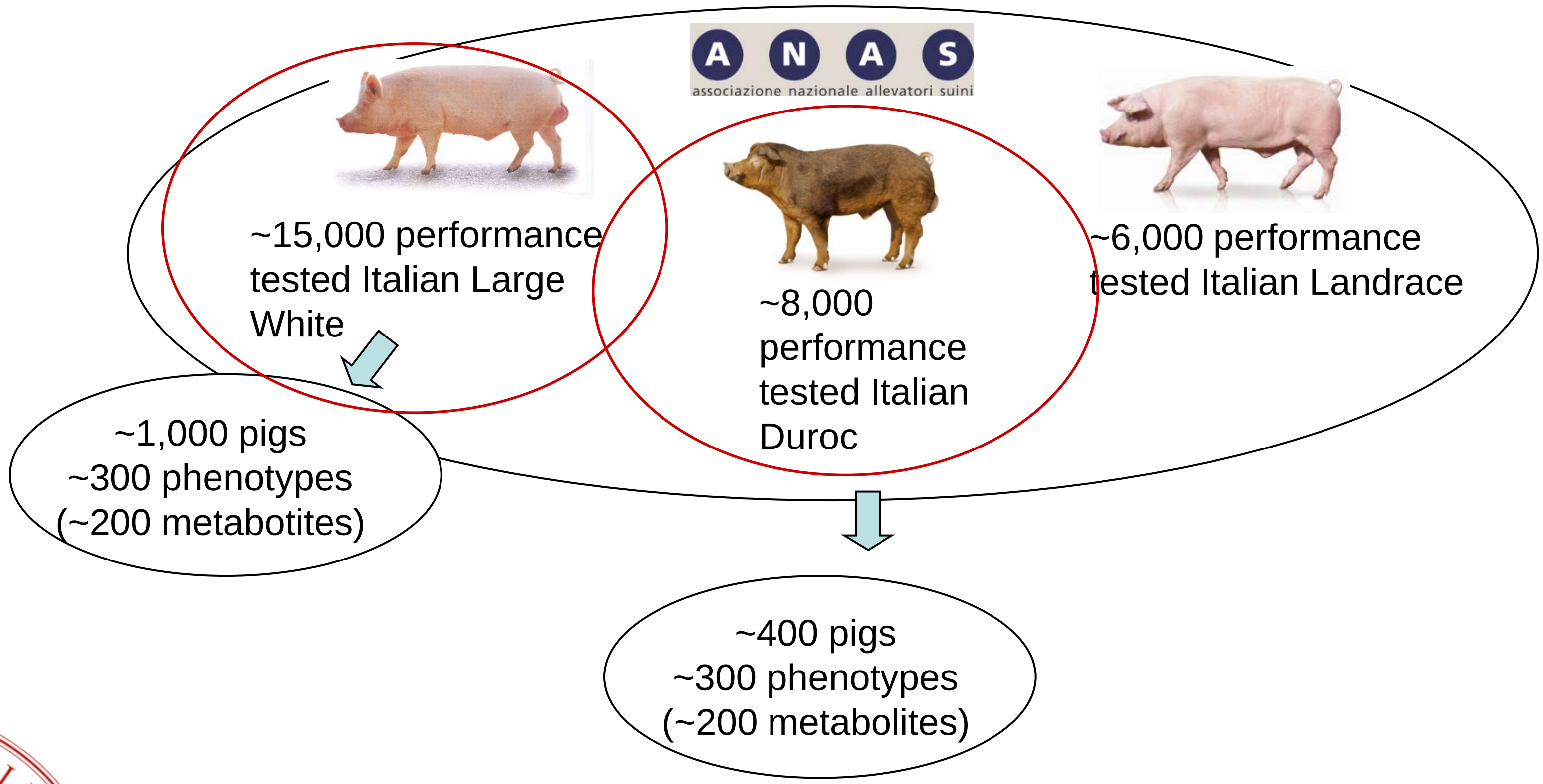
Metabolomics measures all endogenous metabolites of a tissue or body fluid under given conditions (i.e.metabolome)



From Junot et al. 2014

Metabolomics
(Genomics free)

Animal resources



Metabolites ➡ Metabotypes

- Quantitative measure of metabolites
- Phenotypes
  - Molecular phenotypes

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- Quantitative measure of metabolites
- Phenotypes
  - Molecular phenotypes

Targeted metabolomic analysis on blood plasma: ~200 metabolites

Measured by LC-MS/MS or FIA-MS/MS with a AB SCIEX 4500 mass spectrometer

Metabotypes

Metabolite classes	No.	Biological relevance (selected examples)
Acylcarnitines	40	Energy metabolism, fatty acid transport and mitochondrial fatty acid oxidation, ketosis, oxidative stress, mitochondrial membrane damage (apoptosis)
Amino acids	21	Amino acid metabolism, urea cycle, activity of gluconeogenesis and glycolysis, insulin sensitivity/resistance, neurotransmitter metabolism, oxidative stress
Biogenic amines	19	Neurological disorders, cell proliferation, cell cycle progression, DNA stability, oxidative stress
Hexoses	1	Carbohydrate metabolism
Glycerophospholipids	90	
- lysoPhosphatidylcholine acyl – lysoPC a Cx:x	14	Degradation of phospholipids (phospholipase activity), membrane damage, signalling cascades, fatty acid profile
- Phosphatidylcholine diacyl – PC aa Cx:x	38	Dyslipidemia, membrane composition and damage, fatty acid profile, activity of desaturases
- Phosphatidylcholine acyl-alkyl – PC ae Cx:x	38	
Sphingolipids	15	Signalling cascades, membrane damage (e.g. neurodegeneration)

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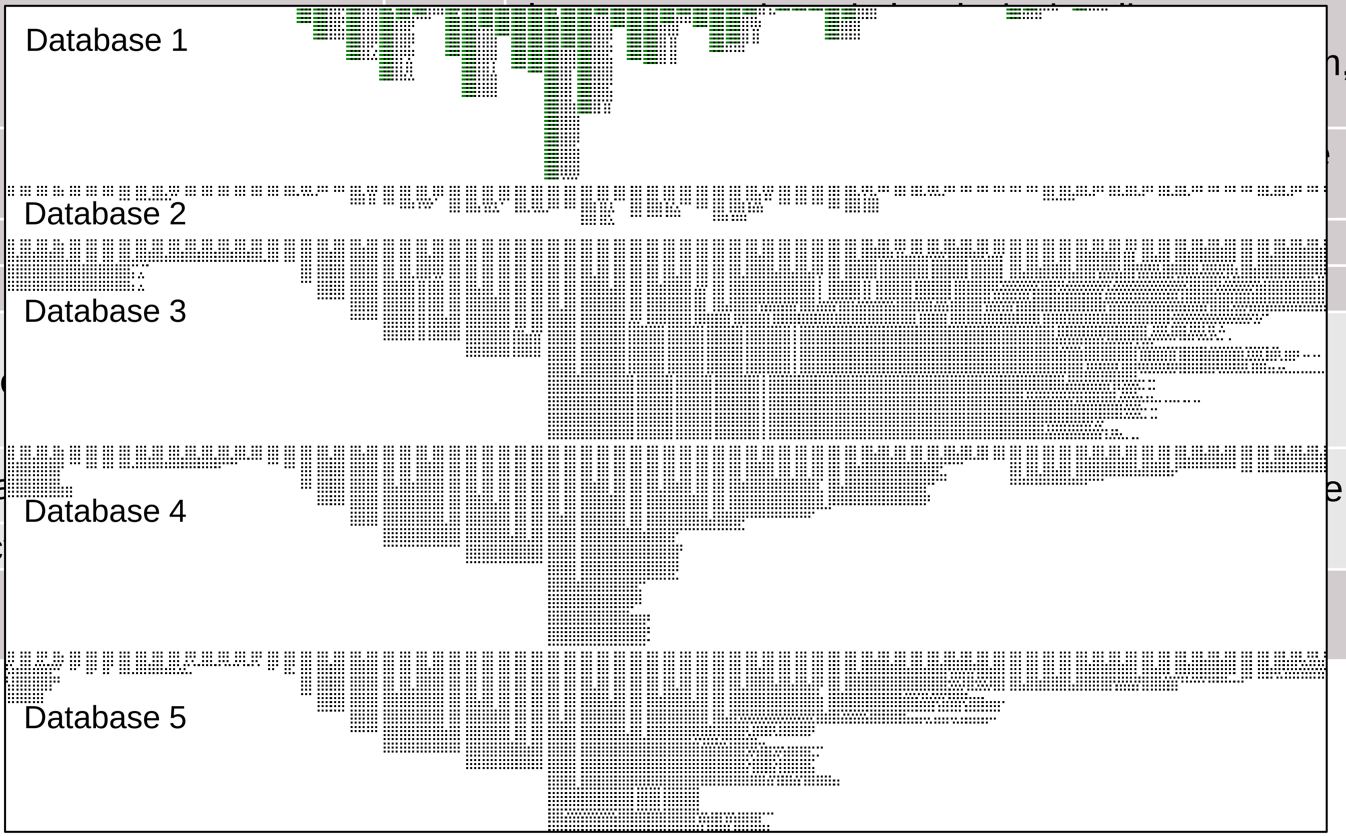
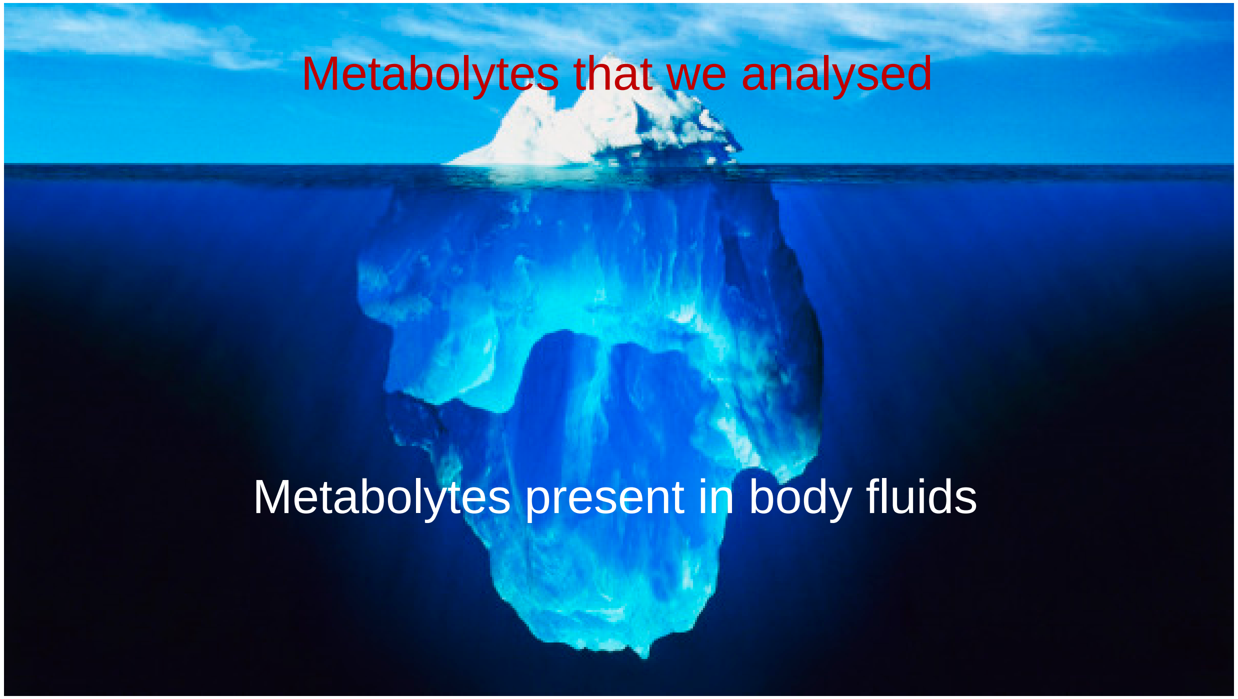
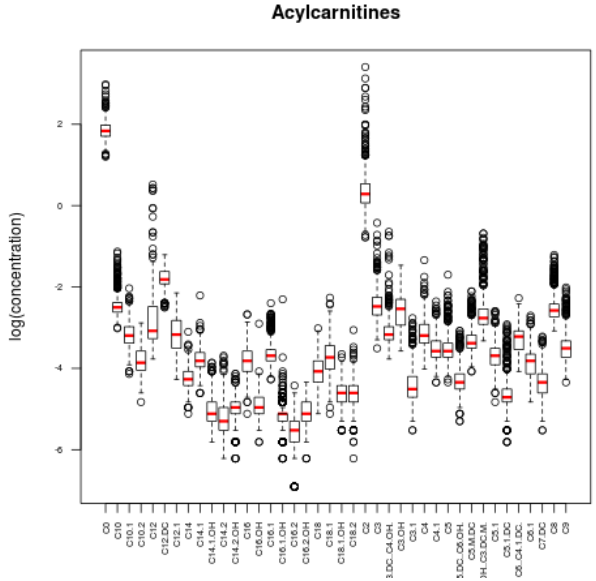
Biogenic amines		
Hexoses		
Glycerophospholipids		
- lysoPhosphatidylcholin		
- Phosphatidylcholine dia		e,
- Phosphatidylcholine ac		
Sphingolipids		

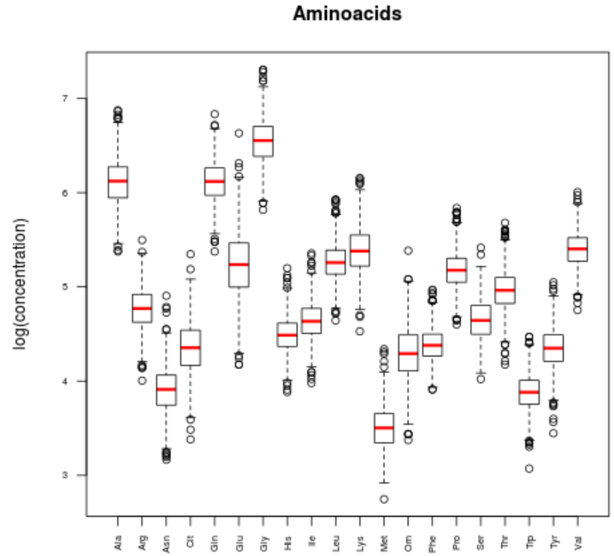


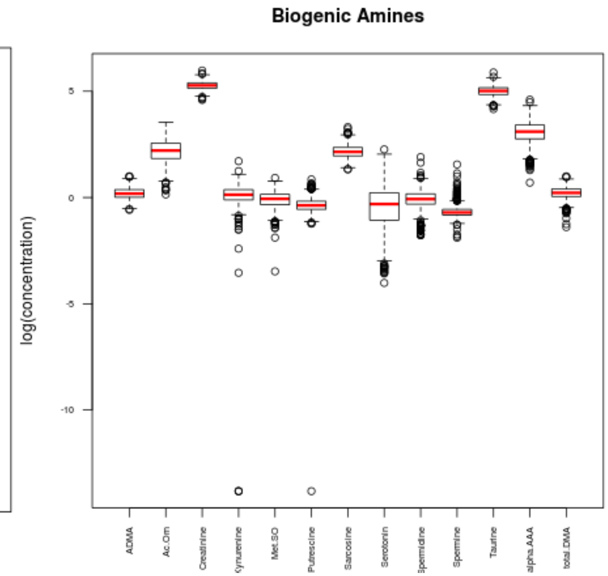
metabotypes

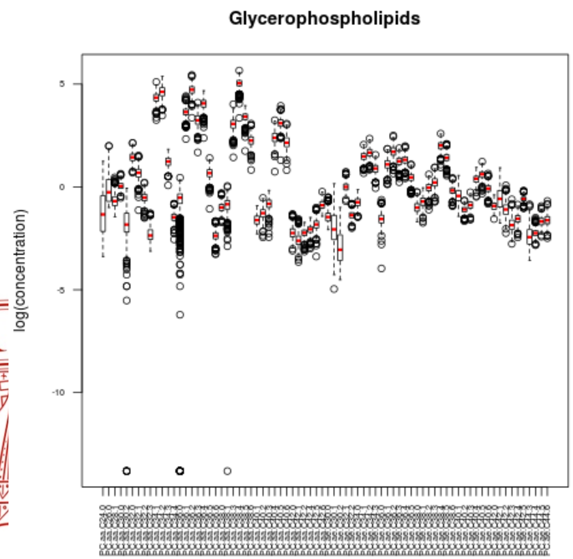

Metabolytes that we analysed
Metabolytes present in body fluids

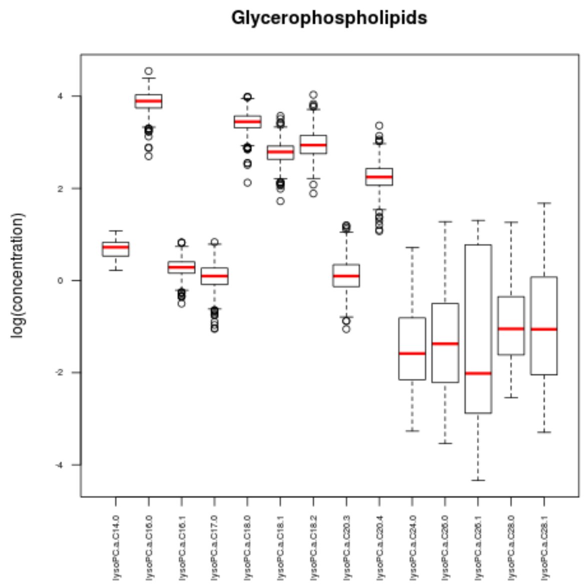
Metabotypes

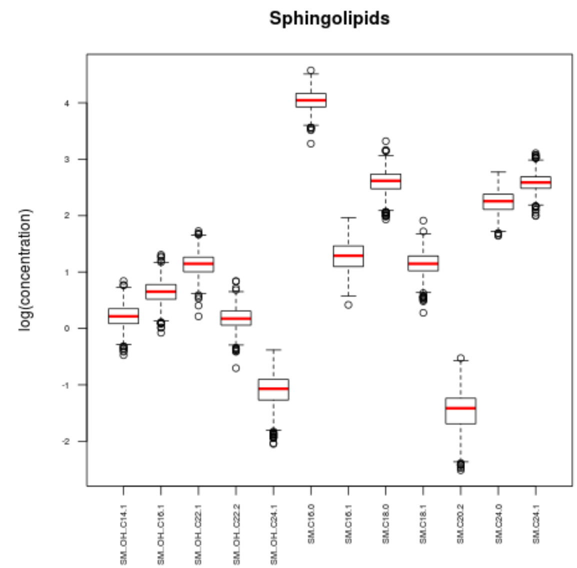






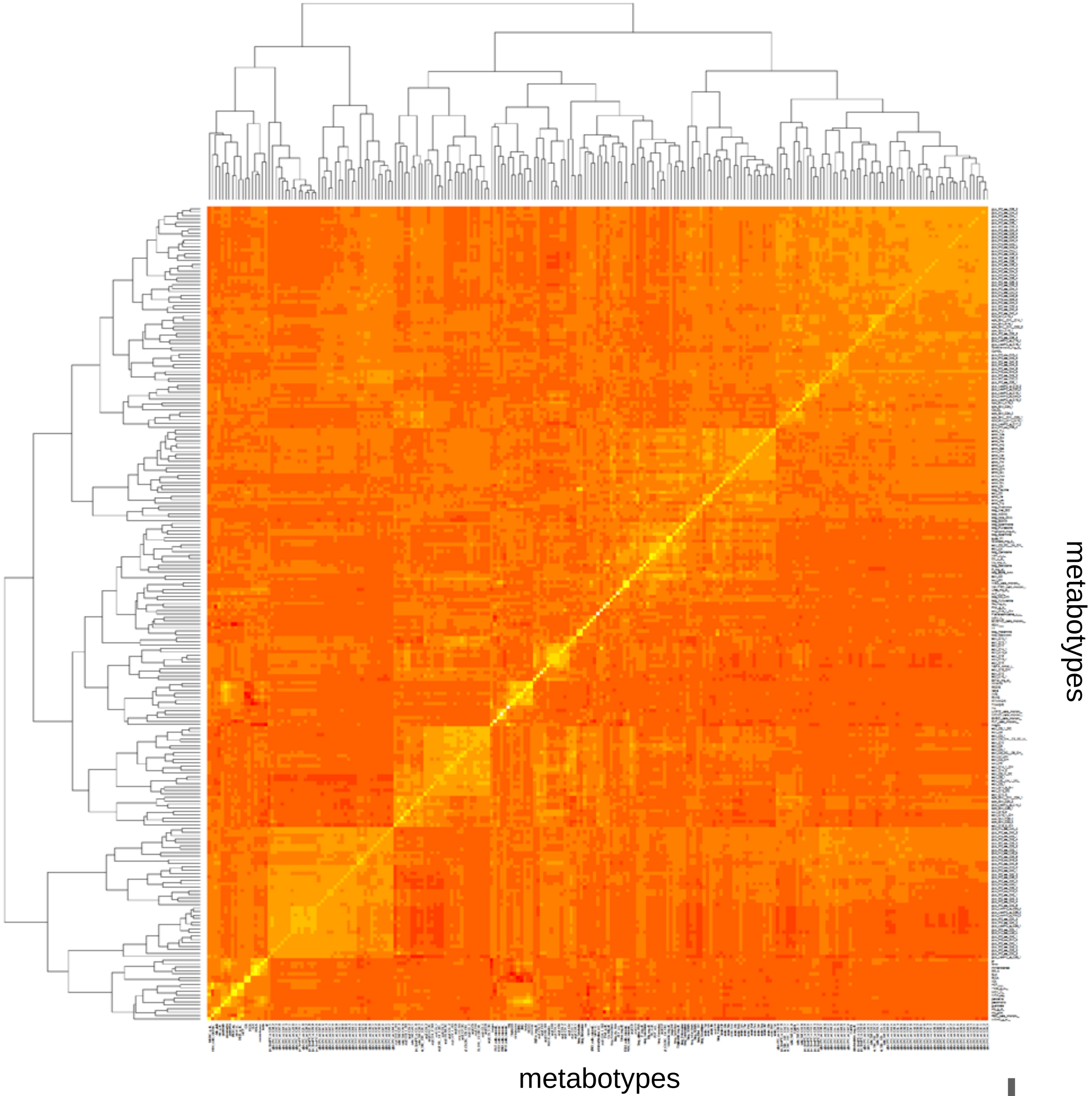




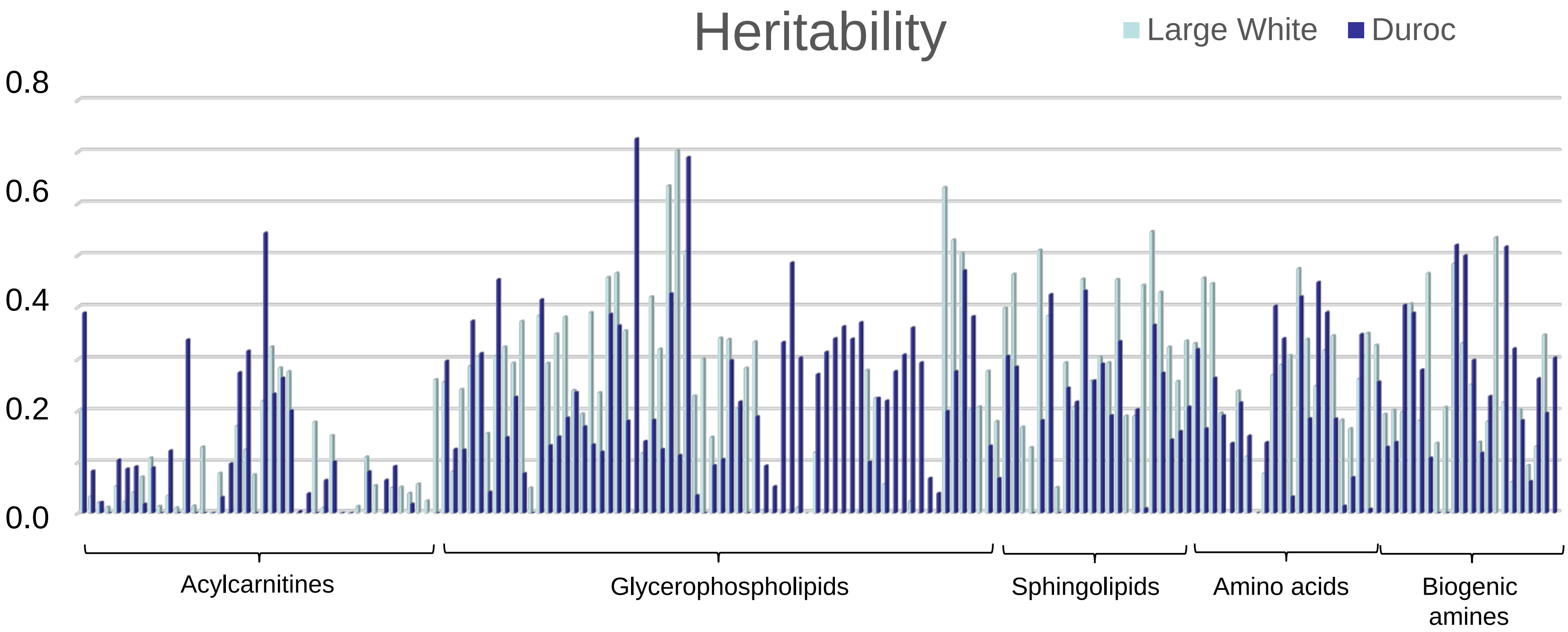


Metabotypes

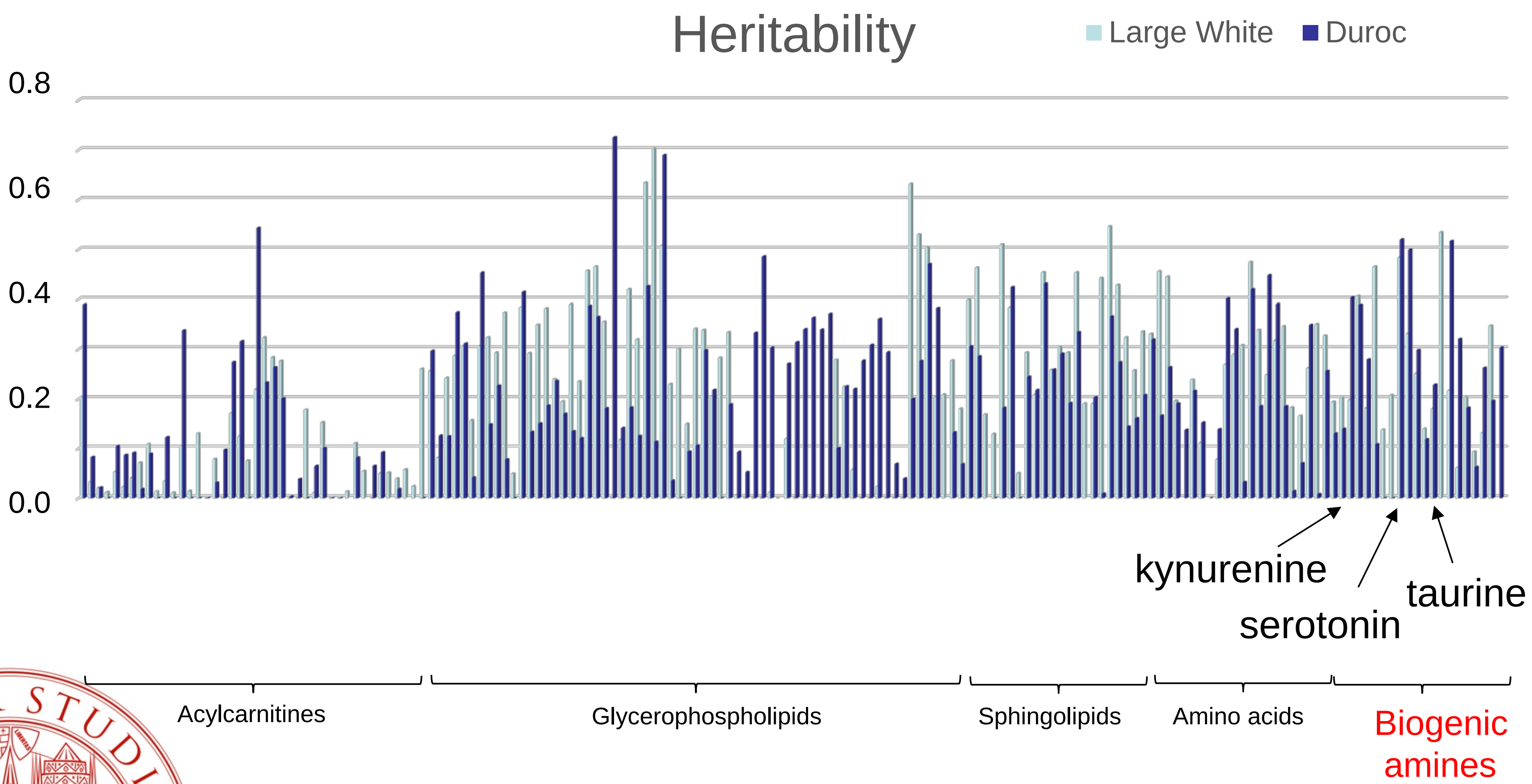
Heatmap with phenotypic correlations



Metabotypes

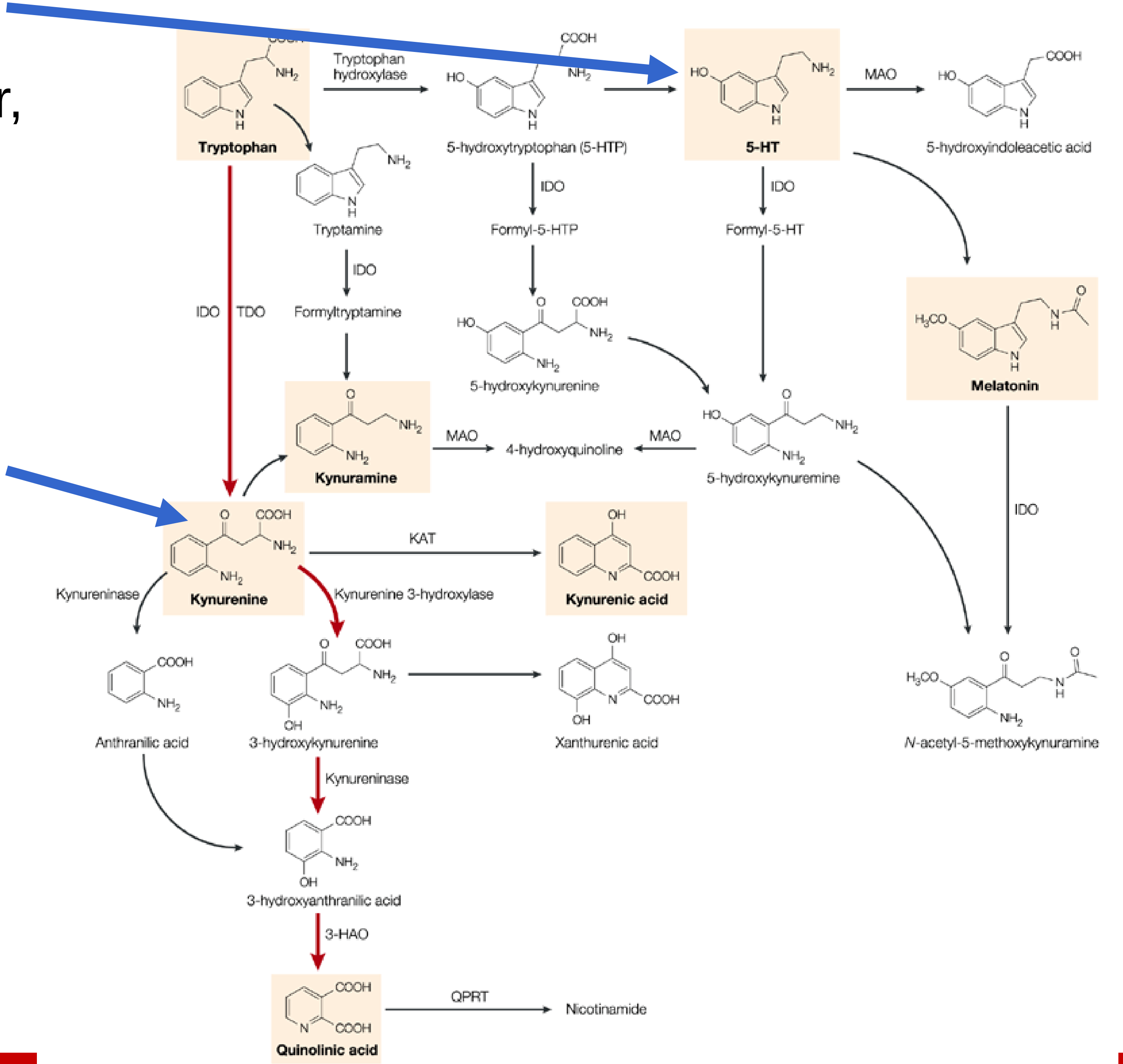


Metabotypes



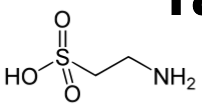
Serotonin: neurotransmitter, known also as the happiness drug

Kynurenine: intermediate metabolite of the tryptophan catabolism

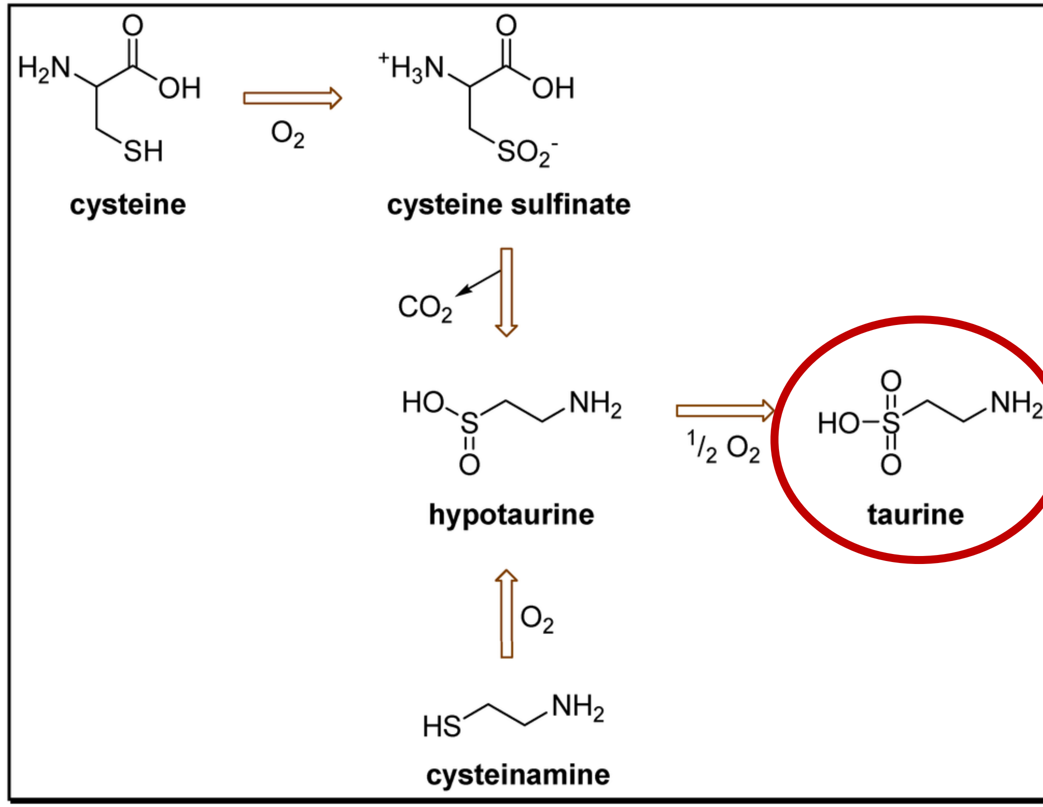


Nature Reviews | Drug Discovery

Taurine:


HO
S
O
O
NH2

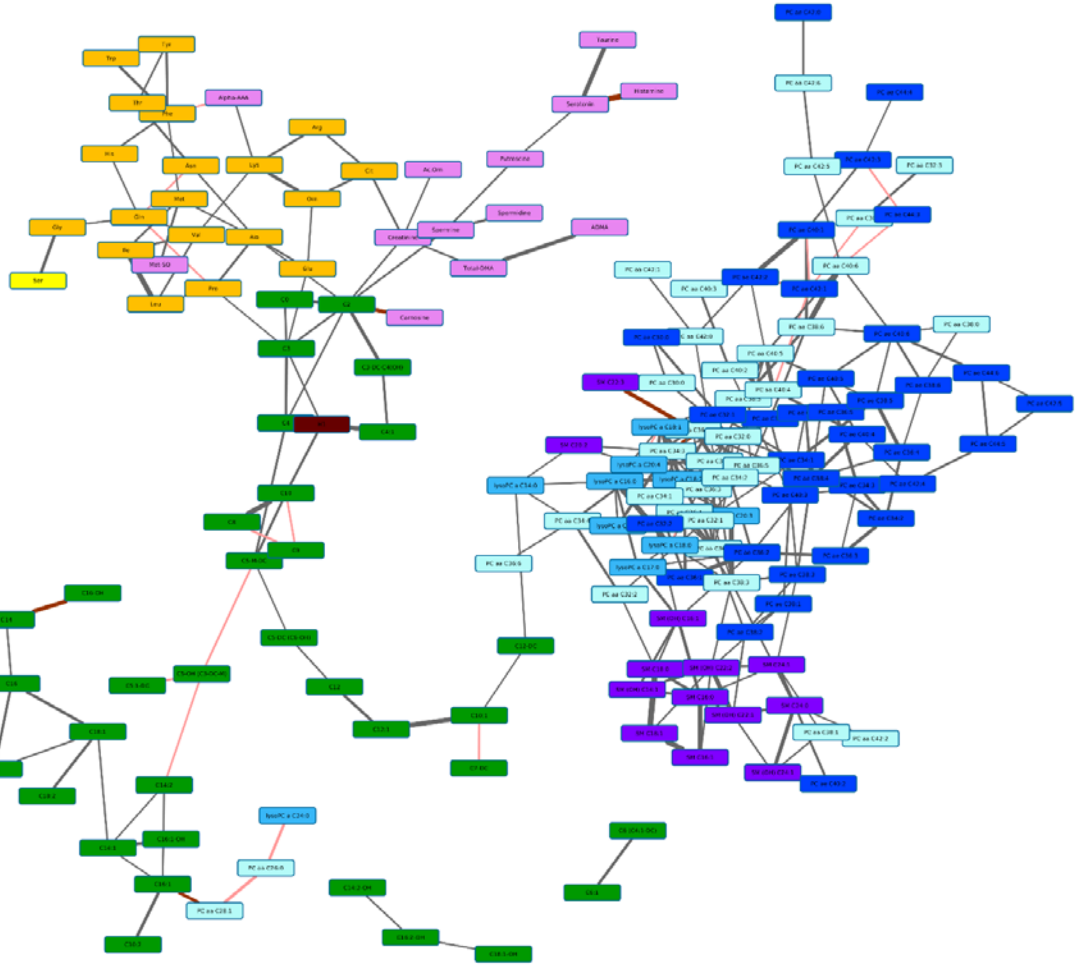

Red
Taurine (0.4%),
Pantothenic acid,
Colours (Caramel,


cysteine
O2
cysteine sulfinate
CO2
hypotaurine
1/2 O2
taurine
O2
cysteinamine

Metabotypes

Gaussian Graphical Model (GGM)

Based on **partial correlation coefficients (PCC):**
pairwise Pearson correlation coefficients conditioned against the correlation with all other metabolites



Metabotypes

Top 8 positive GGM edge weights (i.e. partial correlation coefficients, PCC) in our data set

Metabolite	Metabolite	PCC	
SM C18:0	SM C18:1	0.775	desaturation (1)
lysoPC a C16:0	lysoPC a C18:0	0.731	elongation (1)
SM C16:1	SM C18:1	0.692	elongation (1)
PC aa C38:6	PC aa C40:6	0.689	elongation (1)
PC aa C38:3	lysoPC a C20:3	0.653	phospholipid association ?
PC aa C40:4	PC aa C40:5	0.604	desaturation (1)
lysoPC a C18:2	lysoPC a C20:4	0.600	elong.+ desat. (2)
Serotonin	Taurine	0.594	interaction/regulation ?

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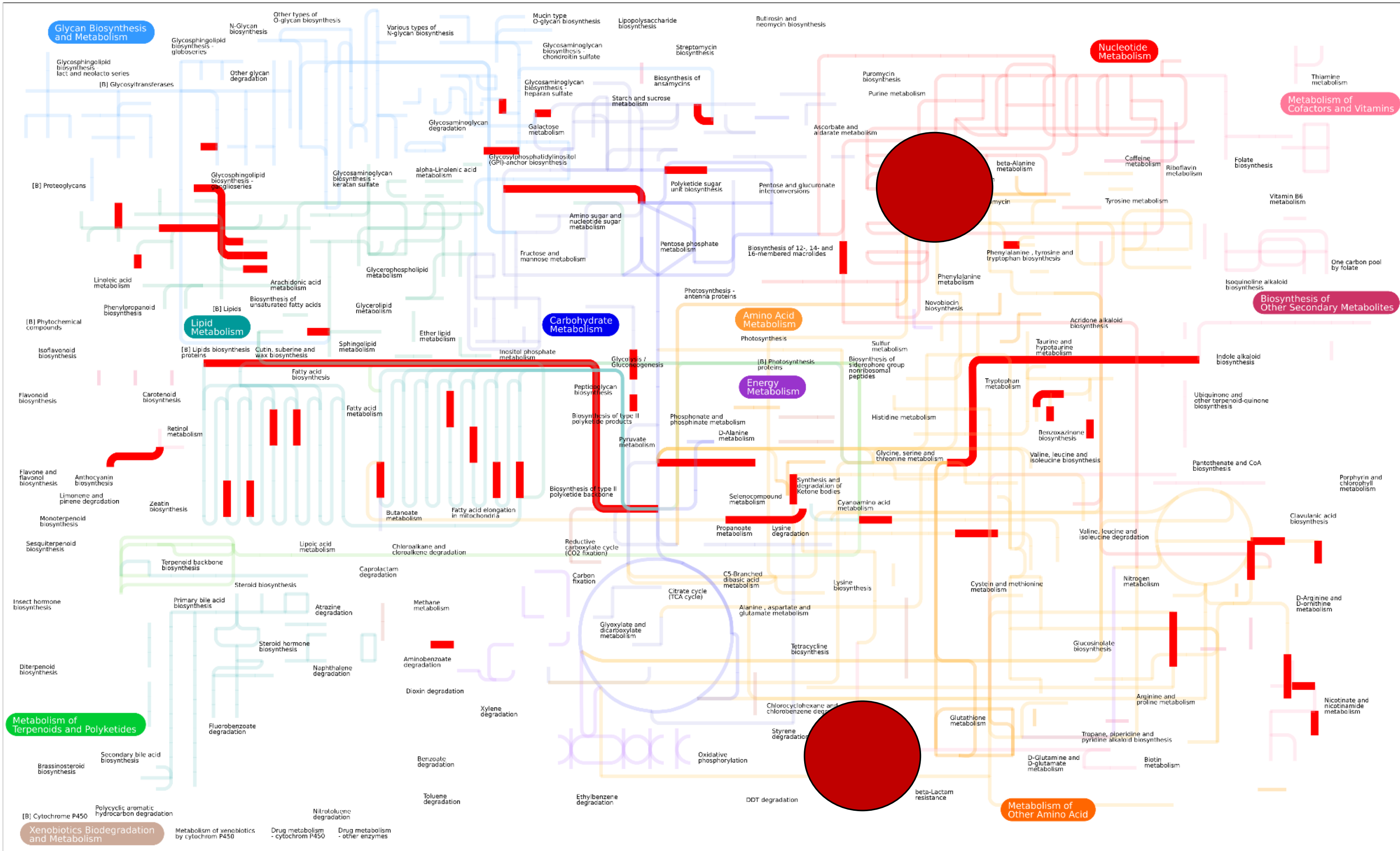
lysoPhosphatidylcholine acyl: lysoPC
Phosphatidylcholine diacyl: PCaa
Phosphatidylcholine acyl-alkyl: PCae
Sphingolipids: SM


Glycan Biosynthesis and Metabolism
Lipid Metabolism
Carbohydrate Metabolism
Amino Acid Metabolism
Energy Metabolism
Nucleotide Metabolism
Metabolism of Cofactors and Vitamins
Biosynthesis of Other Secondary Metabolites
Metabolism of Terpenoids and Polyketides
Xenobiotics Biodegradation and Metabolism
Metabolism of Other Amino Acid
N-Glycan biosynthesis
Other types of O-glycan biosynthesis
Various types of N-glycan biosynthesis
Mucin type O-glycan biosynthesis
Lipopolysaccharide biosynthesis
Butirosin and neomycin biosynthesis
Glycosphingolipid biosynthesis - globoseries
Glycosphingolipid biosynthesis lact and neolacto series
Other glycan degradation
[B] Glycosyltransferases
Glycosaminoglycan biosynthesis - chondroitin sulfate
Streptomycin biosynthesis
Glycosaminoglycan biosynthesis - heparan sulfate
Biosynthesis of ansamycins
Starch and sucrose metabolism
Puromycin biosynthesis
Purine metabolism
Thiamine metabolism
Glycosaminoglycan degradation
Galactose metabolism
Ascorbate and aldarate metabolism
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis
beta-Alanine metabolism
Caffeine metabolism
Riboflavin metabolism
Folate biosynthesis
[B] Proteoglycans
Glycosphingolipid biosynthesis - ganglioseries
Glycosaminoglycan biosynthesis - keratan sulfate
alpha-Linolenic acid metabolism
Polyketide sugar unit biosynthesis
Pentose and glucuronate interconversions
Tyrosine metabolism
Vitamin B6 metabolism
Amino sugar and nucleotide sugar metabolism
Pentose phosphate metabolism
Fructose and mannose metabolism
Biosynthesis of 12-, 14- and 16-membered macrolides
Phenylalanine, tyrosine and tryptophan biosynthesis
One carbon pool by folate
Linoleic acid metabolism
Arachidonic acid metabolism
Glycerophospholipid metabolism
Phenylalanine metabolism
Isoquinoline alkaloid biosynthesis
Phenylpropanoid biosynthesis
[B] Lipids
Biosynthesis of unsaturated fatty acids
Glycerolipid metabolism
Photosynthesis - antenna proteins
Novobiocin biosynthesis
[B] Phytochemical compounds
Ether lipid metabolism
Acridone alkaloid biosynthesis
Sphingolipid metabolism
Photosynthesis
Isoflavonoid biosynthesis
[B] Lipids biosynthesis proteins
Cutin, suberine and wax biosynthesis
Inositol phosphate metabolism
Glycolysis / Gluconeogenesis
[B] Photosynthesis proteins
Sulfur metabolism
Biosynthesis of siderophore group nonribosomal peptides
Taurine and hypotaurine metabolism
Indole alkaloid biosynthesis
Fatty acid biosynthesis
Flavonoid biosynthesis
Carotenoid biosynthesis
Peptidoglycan biosynthesis
Tryptophan metabolism
Ubiquinone and other terpenoid-quinone biosynthesis
Fatty acid metabolism
Biosynthesis of type II polyketide products
Phosphonate and phosphinate metabolism
Histidine metabolism
Retinol metabolism
D-Alanine metabolism
Benzoxazinone biosynthesis
Pyruvate metabolism
Glycine, serine and threonine metabolism
Valine, leucine and isoleucine biosynthesis
Pantothenate and CoA biosynthesis
Flavone and flavonol biosynthesis
Anthocyanin biosynthesis
Porphyrin and chlorophyll metabolism
Biosynthesis of type II polyketide backbone
Synthesis and degradation of Ketone bodies
Limonene and pinene degradation
Zeatin biosynthesis
Selenocompound metabolism
Cyanoamino acid metabolism
Butanoate metabolism
Fatty acid elongation in mitochondria
Monoterpenoid biosynthesis
Clavulanic acid biosynthesis
Propanoate metabolism
Lysine degradation
Valine, leucine and isoleucine degradation
Sesquiterpenoid biosynthesis
Lipoic acid metabolism
Chloroalkane and chloroalkene degradation
Reductive carboxylate cycle (CO2 fixation)
Terpenoid backbone biosynthesis
Caprolactam degradation
Carbon fixation
C5-Branched dibasic acid metabolism
Lysine biosynthesis
Cystein and methionine metabolism
Nitrogen metabolism
Steroid biosynthesis
Citrate cycle (TCA cycle)
D-Arginine and D-ornithine metabolism
Insect hormone biosynthesis
Primary bile acid biosynthesis
Atrazine degradation
Methane metabolism
Alanine, aspartate and glutamate metabolism
Glyoxylate and dicarboxylate metabolism
Steroid hormone biosynthesis
Tetracycline biosynthesis
Glucosinolate biosynthesis
Aminobenzoate degradation
Diterpenoid biosynthesis
Naphthalene degradation
Dioxin degradation
Arginine and proline metabolism
Xylene degradation
Chlorocyclohexane and chlorobenzene degradation
Nicotinate and nicotinamide metabolism
Glutathione metabolism
Fluorobenzoate degradation
Styrene degradation
Tropane, piperidine and pyridine alkaloid biosynthesis
Secondary bile acid biosynthesis
Oxidative phosphorylation
D-Glutamine and D-glutamate metabolism
Biotin metabolism
Brassinosteroid biosynthesis
Benzoate degradation
Toluene degradation
Ethylbenzene degradation
DDT degradation
beta-Lactam resistance
[B] Cytochrome P450
Polycyclic aromatic hydrocarbon degradation
Nitrotoluene degradation
Metabolism of xenobiotics by cytochrome P450
Drug metabolism - cytochrome P450
Drug metabolism - other enzymes

Metabotypes

Top 8 positive GGM edge weights (i.e. partial correlation coefficients, PCC) in our data set

Metabolite	Metabolite	PCC	
SM C18:0	SM C18:1	[illegible]	[illegible]aturation (1)
lysoPC a C16:0	lysoP[illegible]	[illegible]	[illegible]gation (1)
SM C16:1	[illegible]	[illegible]	[illegible]ngation (1)
PC a[illegible]	[illegible]	[illegible]	elongation (1)
[illegible]	[illegible]	0.653	phospholipid association ?
[illegible]	[illegible] aa C40:5	0.604	desaturation (1)
[illegible]	lysoPC a C20:4	0.600	elong.+ desat. (2)
[illegible]otonin	Taurine	0.594	interaction/regulation ?

…..
….
…

Reconstruction of biochemical pathways without any direct biochemical experiment

lysoPhosphatidylcholine acyl: lysoPC
Phosphatidylcholine diacyl: PCaa
Phosphatidylcholine acyl-alkyl: PCae
Sphingolipids: SM

How can we link potential
«welfare bio-markers»
with genomics information?

Metabolomics

+

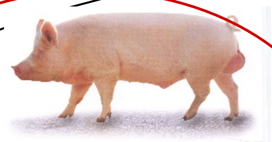
Genomics

Running title:

Searching the happiness genes in pigs …

Animal resources

ANAS
associazione nazionale allevatori suini



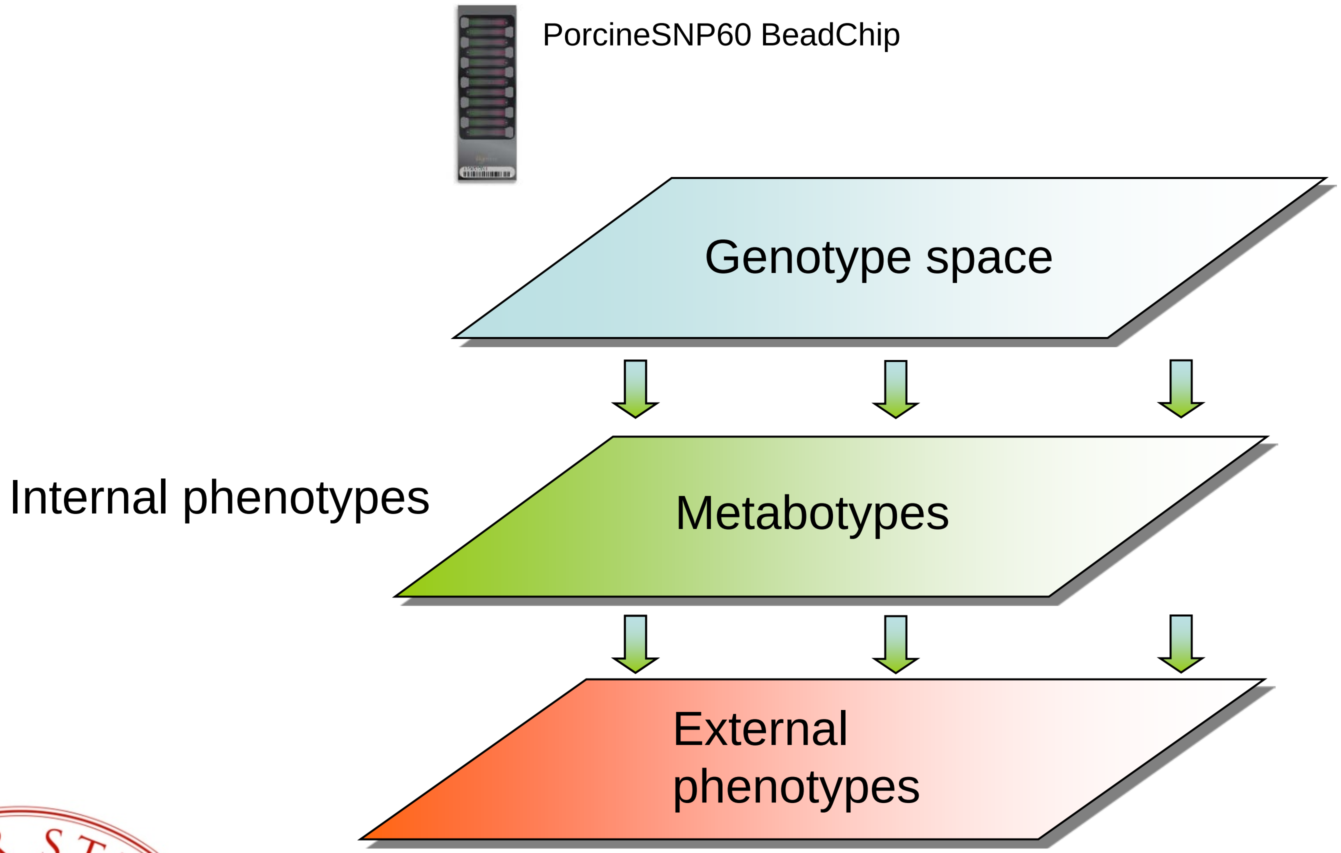
~15,000 performance tested Italian Large White

~8,000 performance tested Italian Duroc

~6,000 performance tested Italian Landrace

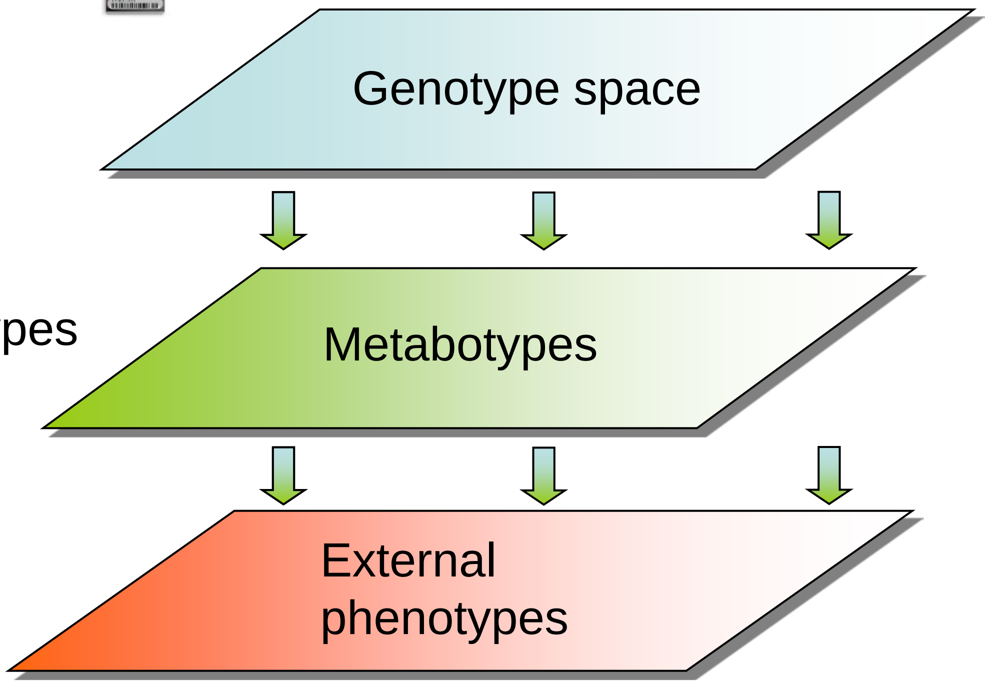
~1,000 pigs
~300 phenotypes
(~200 metabotypes)

~400 pigs
~300 phenotypes
(~200 metabotypes)


PorcineSNP60 BeadChip
Genotype space
Internal phenotypes
Metabotypes
External
phenotypes

PorcineSNP60 BeadChip

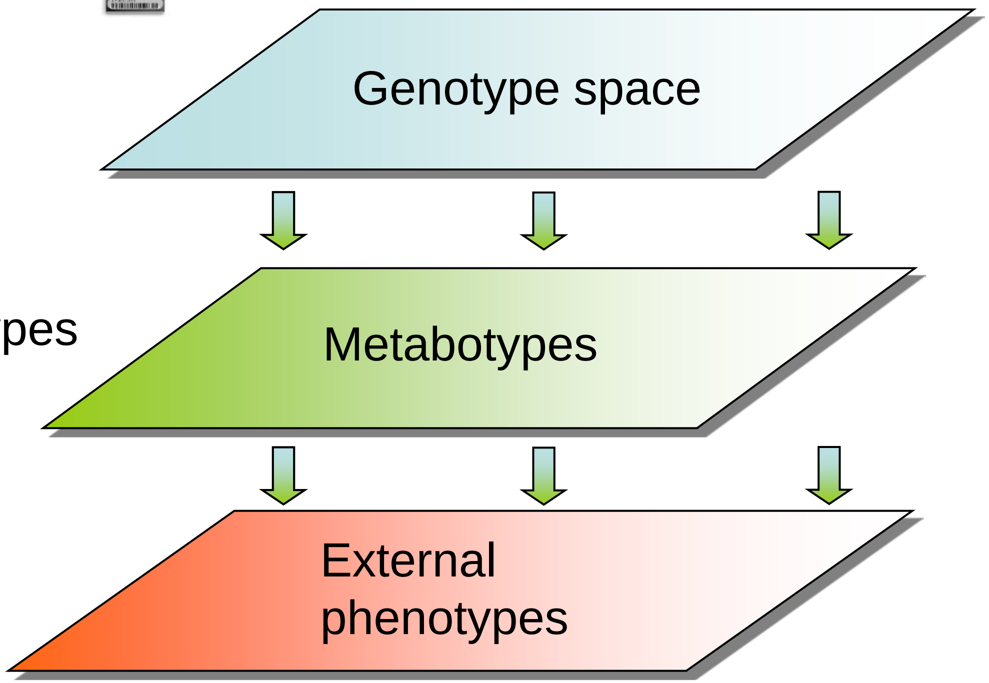
Internal phenotypes



~200 metabotypes

PorcineSNP60 BeadChip

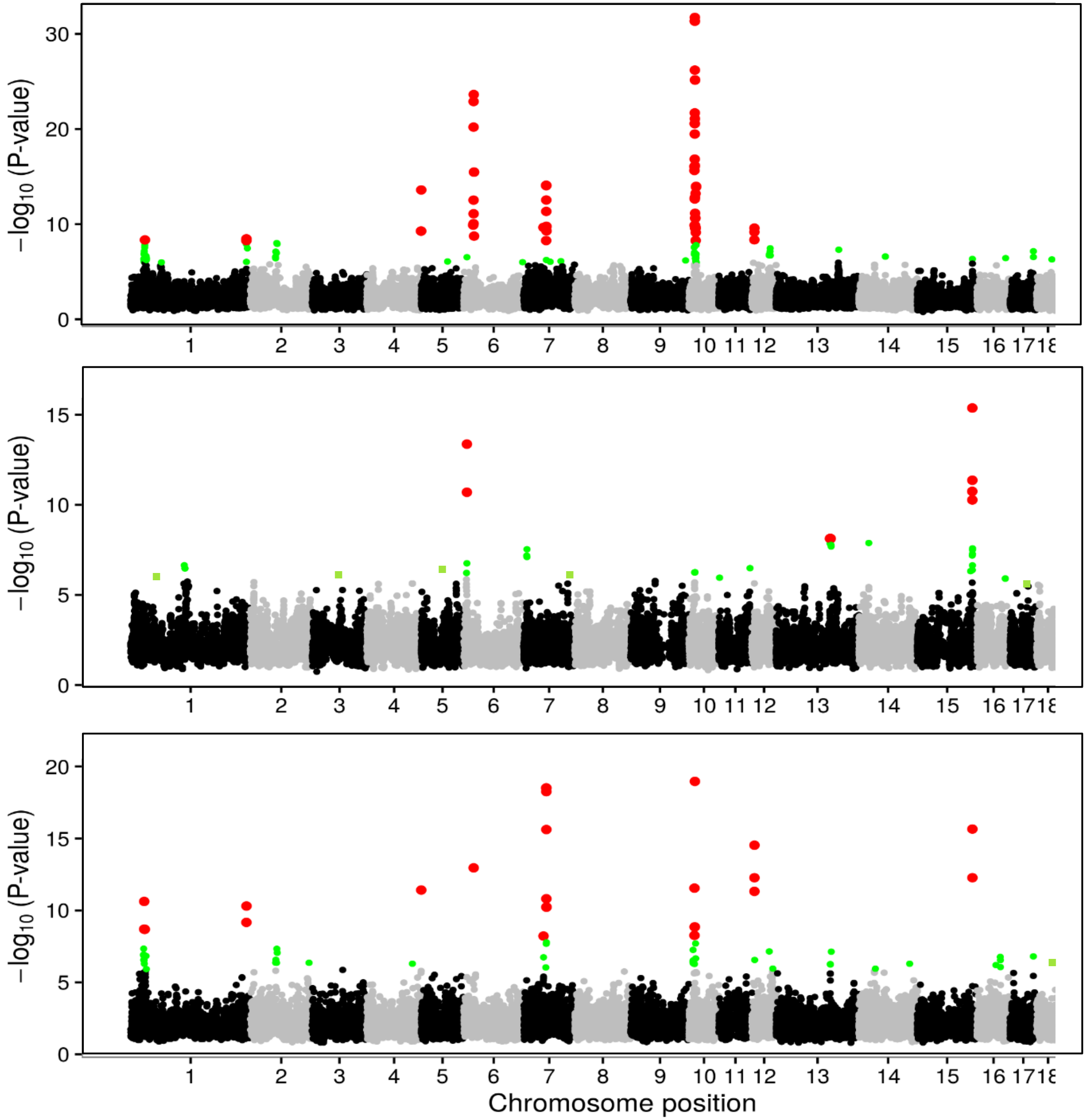
Internal phenotypes



~200 metabotypes

200 x 200 = 40000 ratios

Single

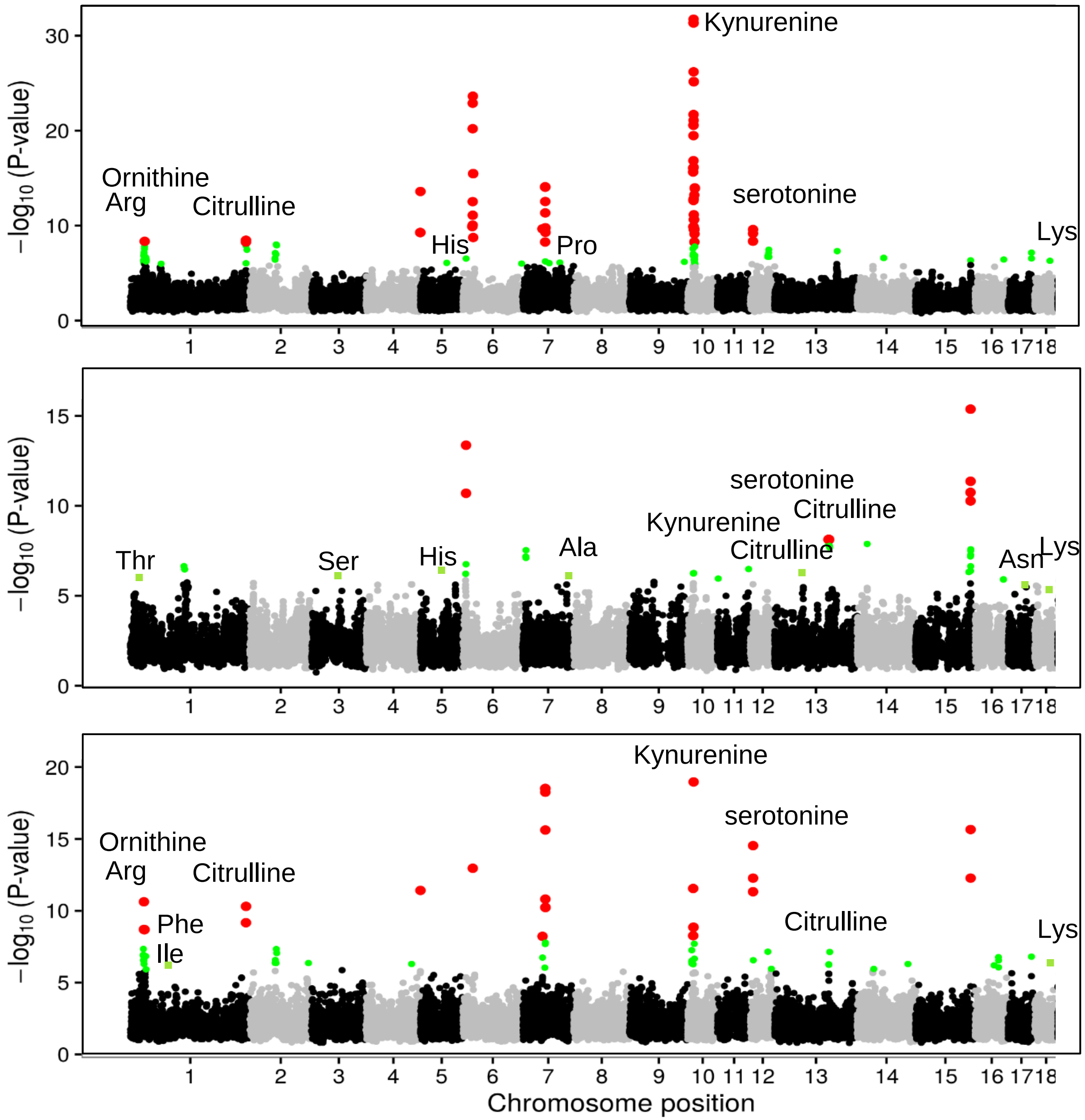


Italian Large White

Italian Duroc

Combined

Single

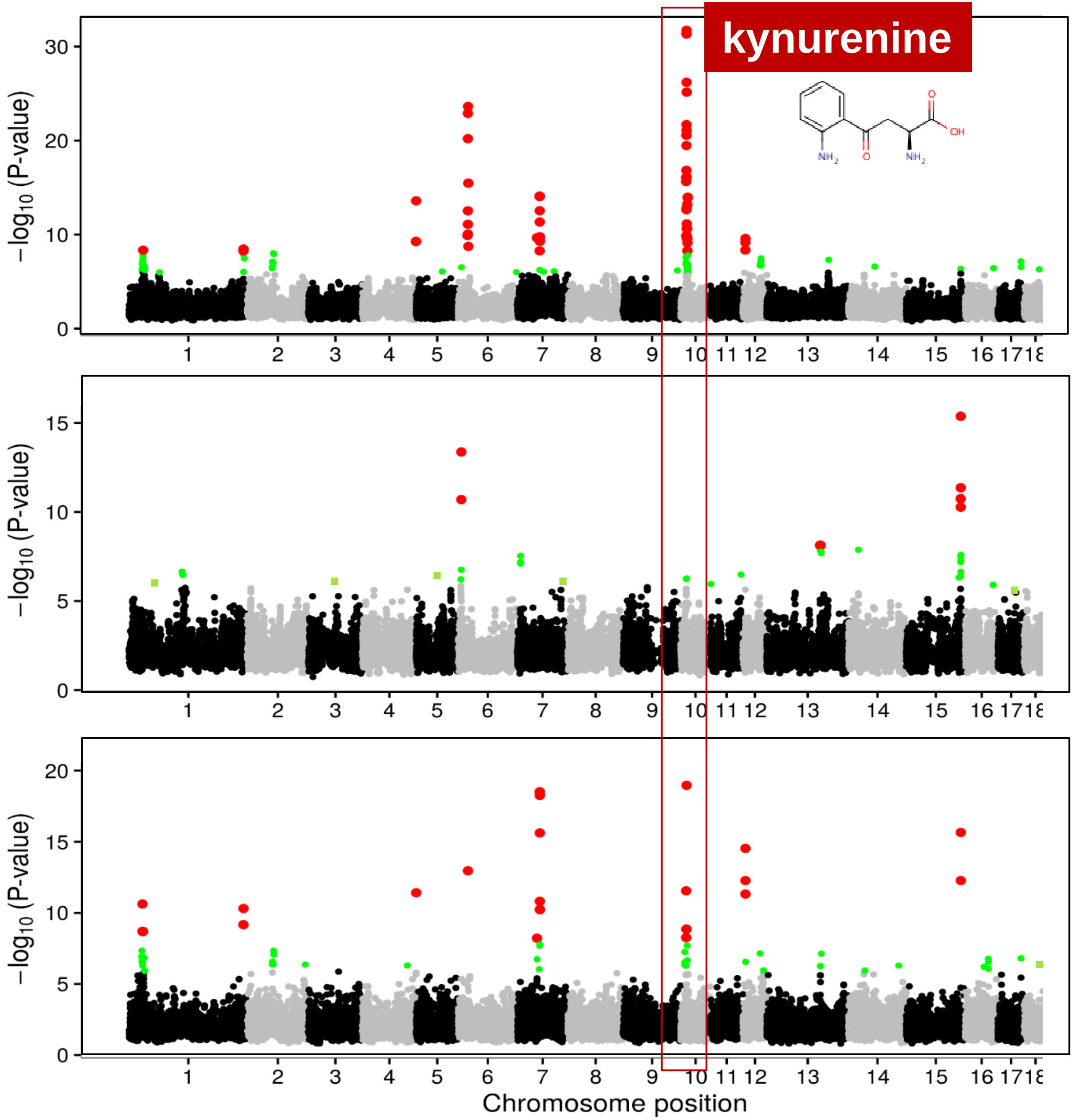


Italian Large White

Italian Duroc

Combined

Single

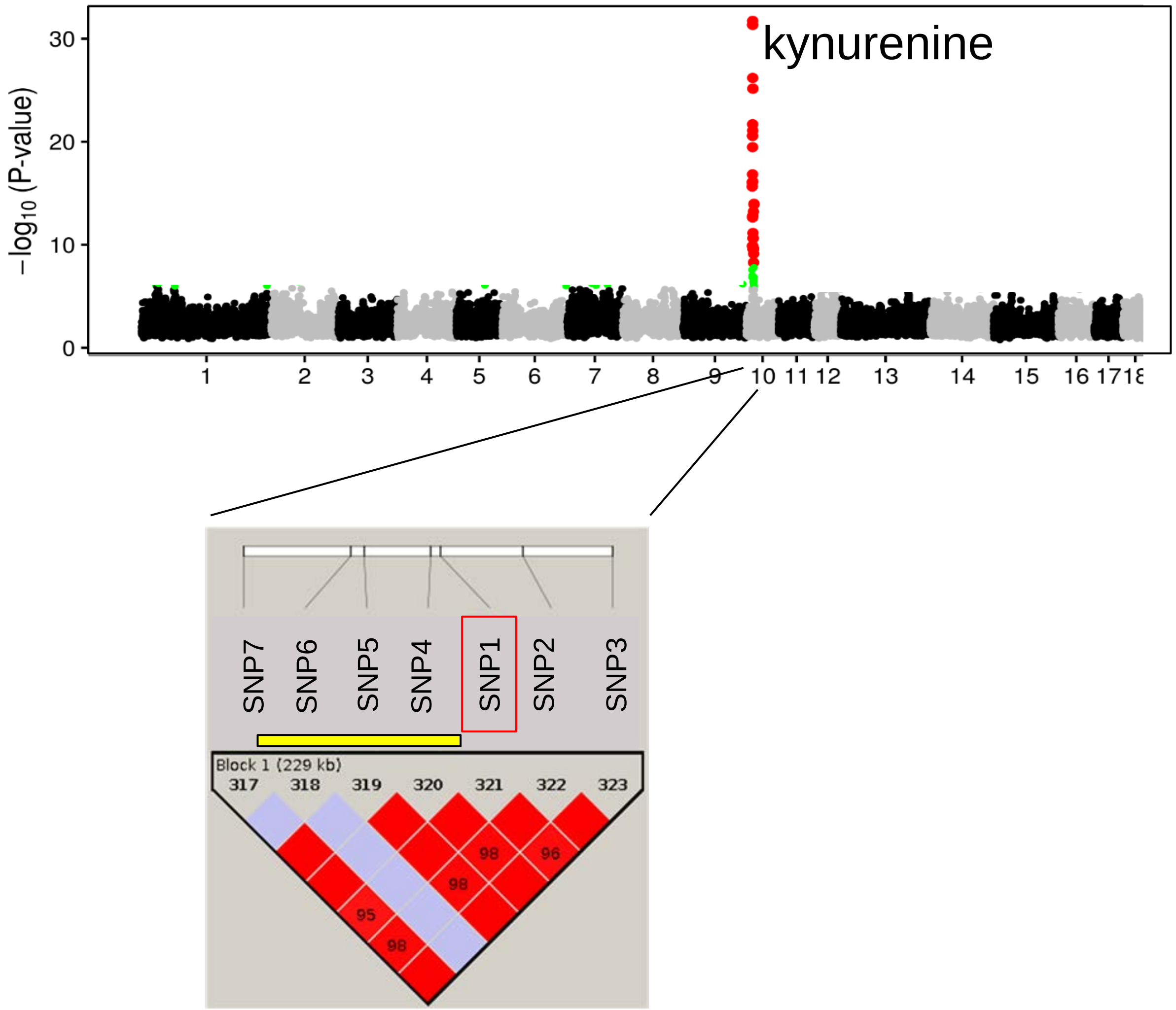


Italian Large White

Italian Duroc

Combined

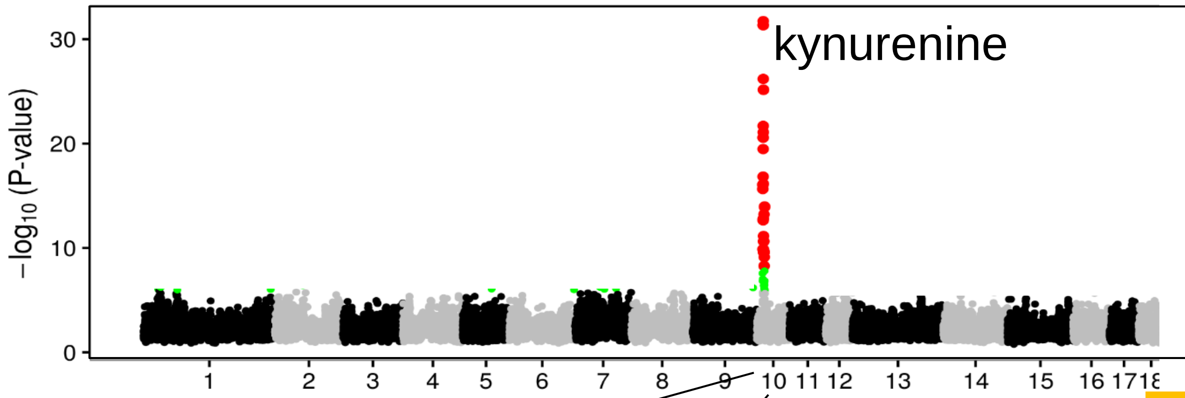
Single



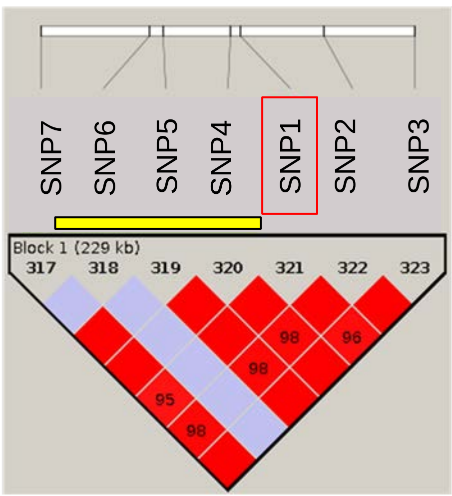
Italian Large White

Kynurenine 3-monooxygenase (KMO)

Single

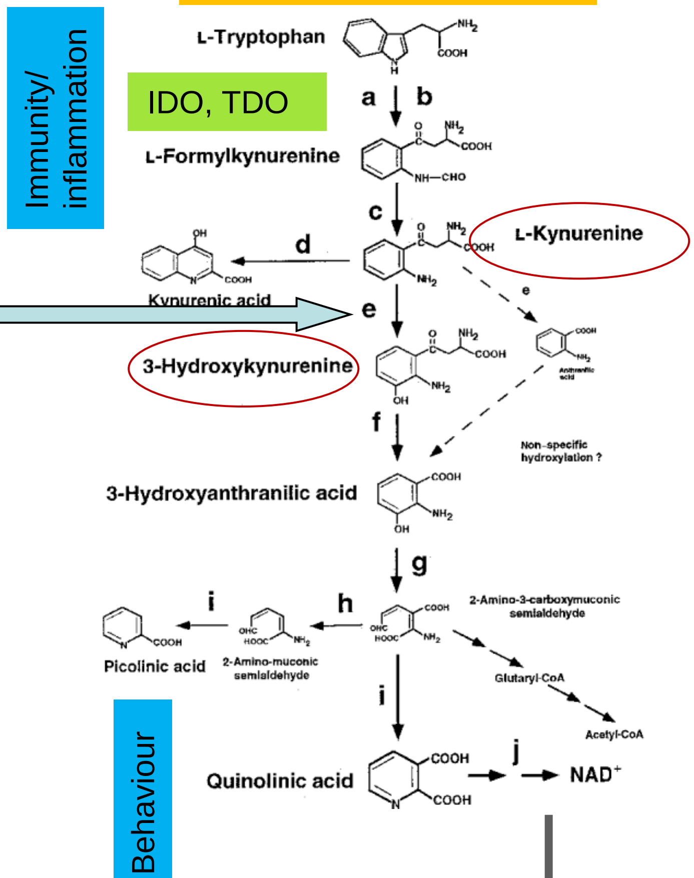


Italian Large White

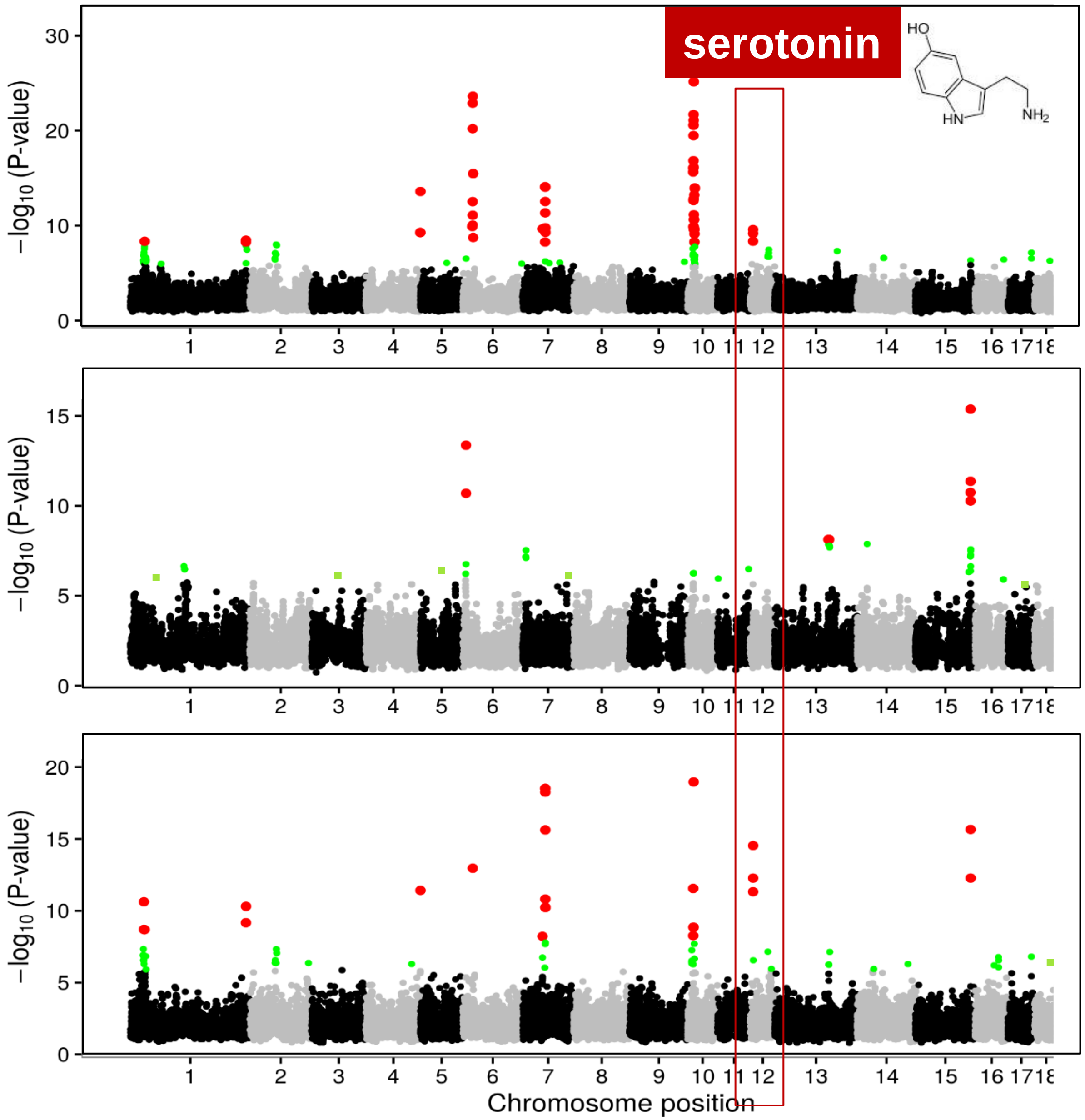


Kynurenine
3-monooxygenase
(KMO)

Tryptophan catabolism



Single

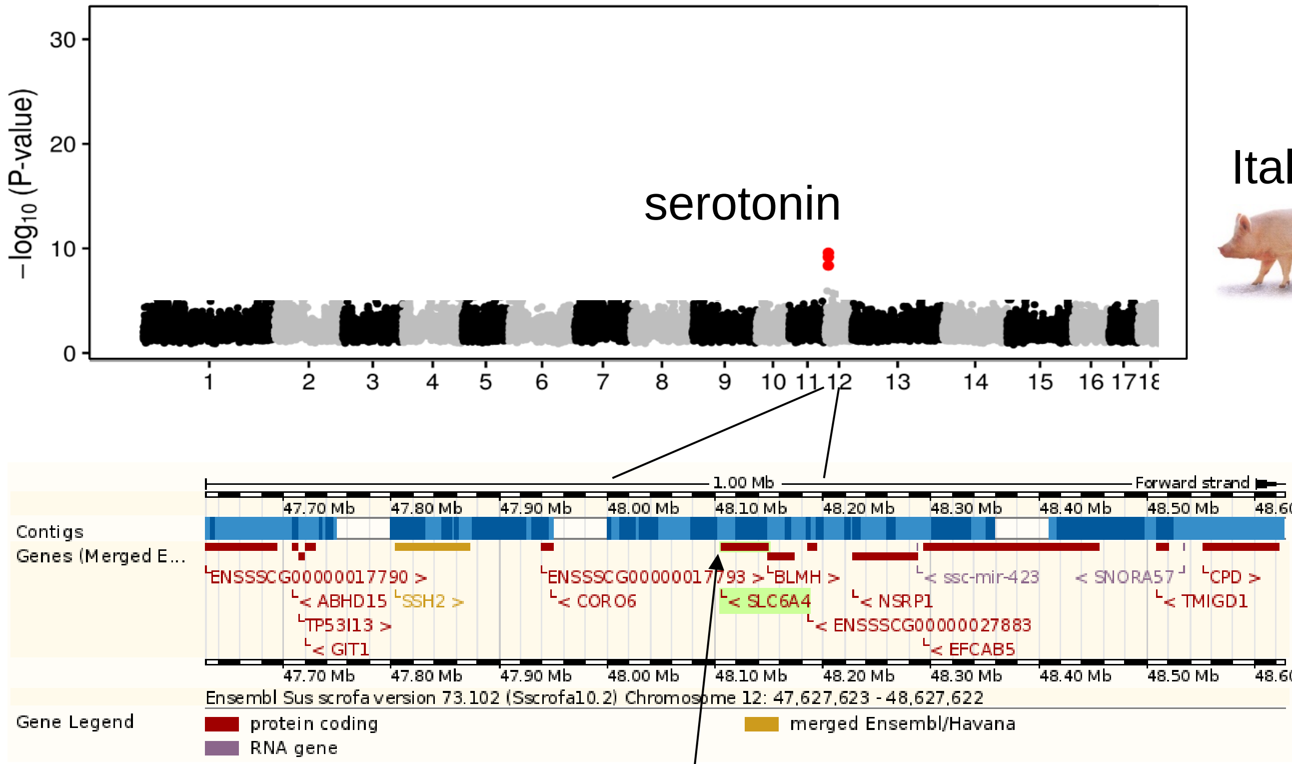


Italian Large White

Italian Duroc

Combined

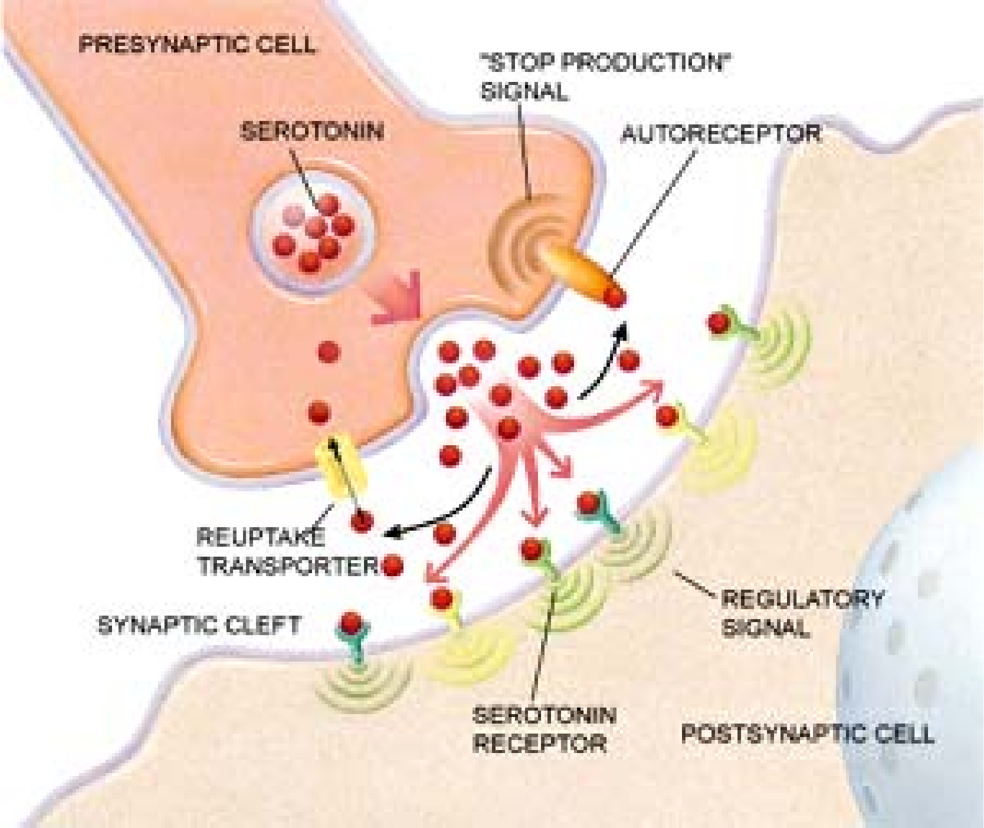
Single



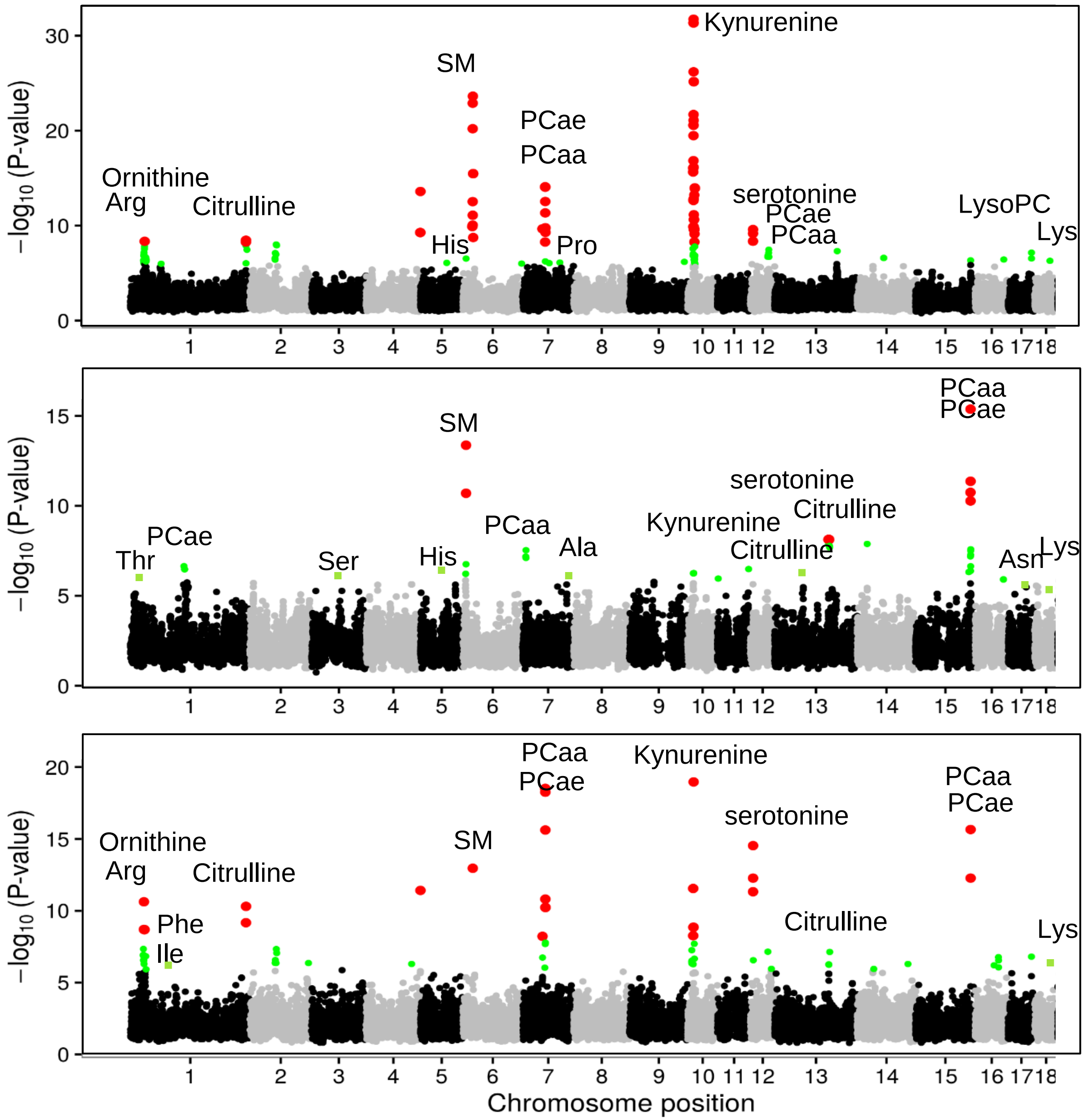
Italian Large White

SLC6A4

Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4


PRESYNAPTIC CELL
"STOP PRODUCTION"
SIGNAL
SEROTONIN
AUTORECEPTOR
REUPTAKE
TRANSPORTER
SYNAPTIC CLEFT
REGULATORY
SIGNAL
SEROTONIN
RECEPTOR
POSTSYNAPTIC CELL

Single

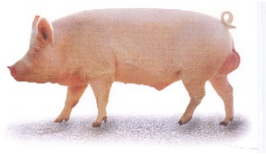


Italian Large White

Italian Duroc

Combined


Our omics study in pigs

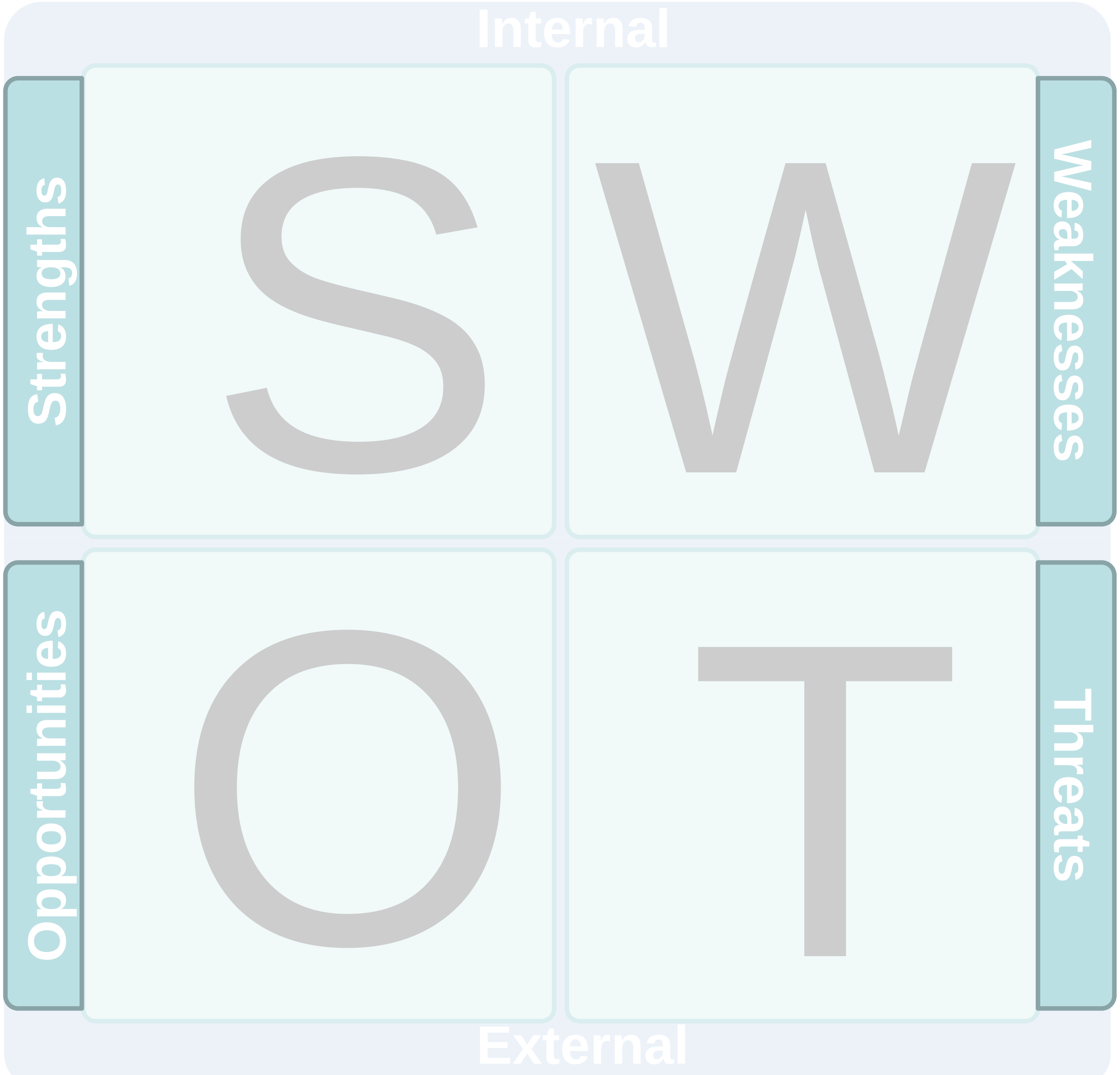


- Hypothesis generating

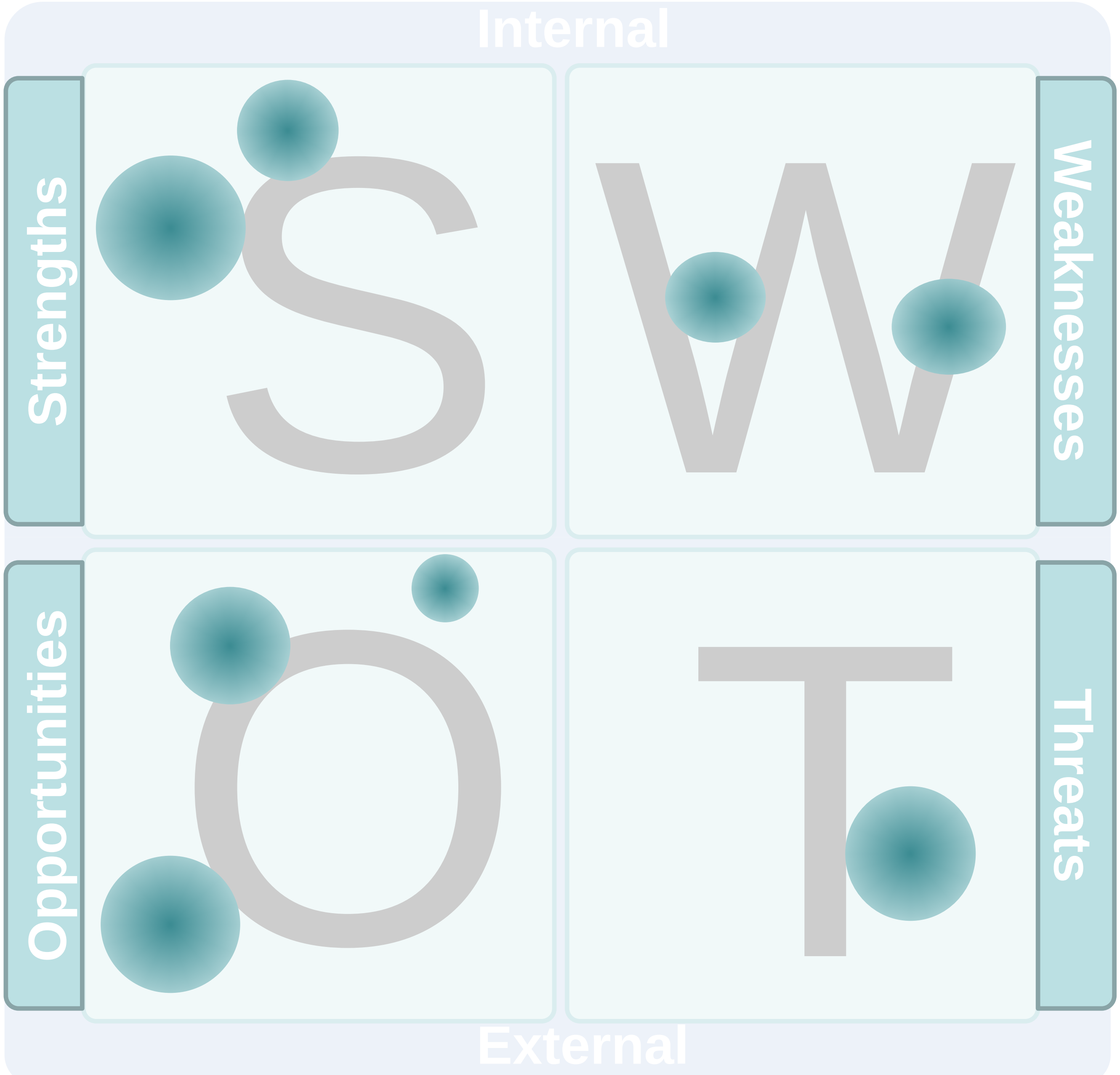
Nutrigenetics
Behaviour
…

Conclusions

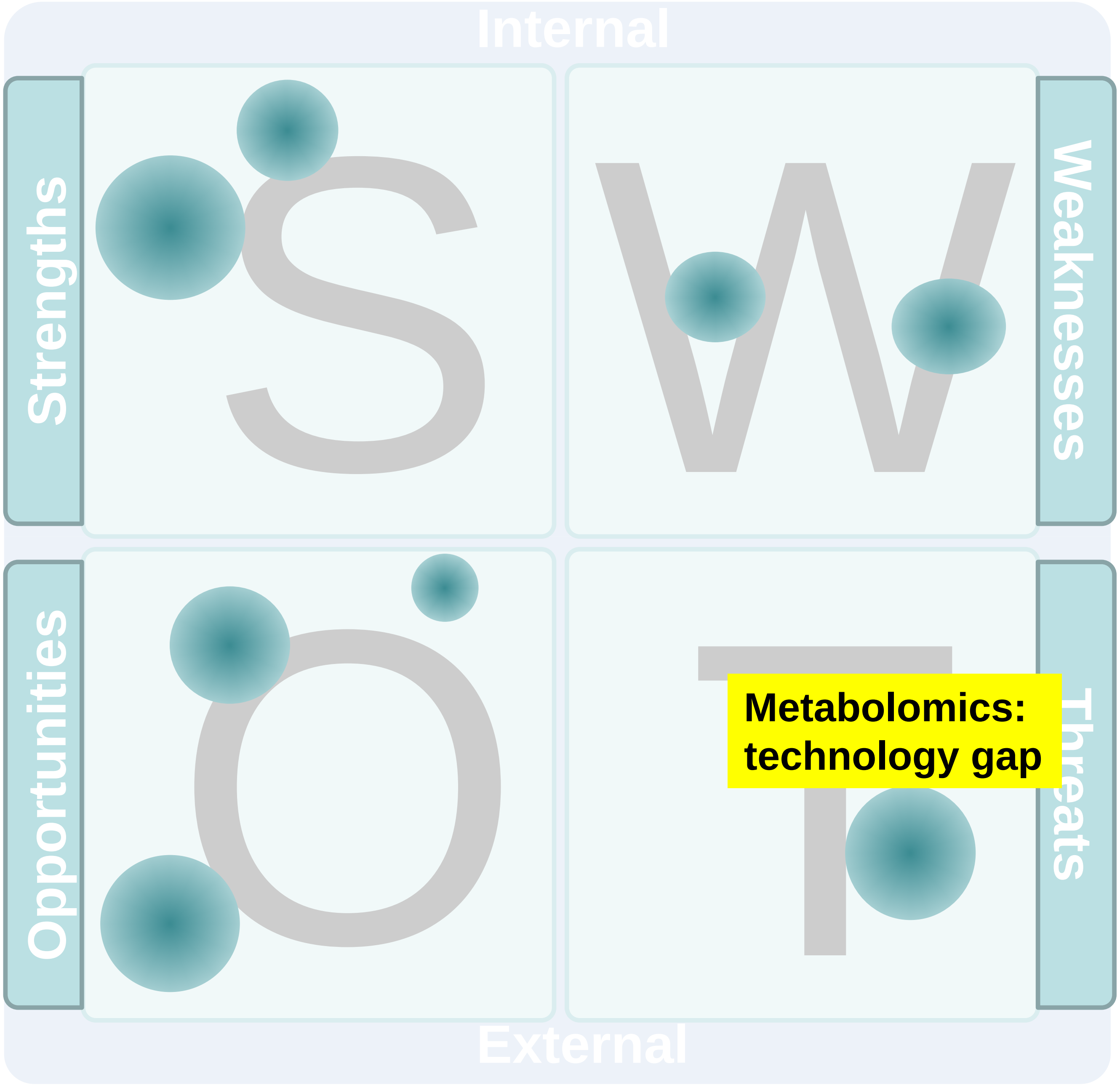
Conclusions



Conclusions



Conclusions



Conclusions

Internal

Strengths – S

Weaknesses – W

Limited number of metabolites

Data analysis: New approaches needed

Opportunities – O

Threats – T

Metabolomics: technology gap

External

Conclusions

Internal

Strengths

Close link to genomics

Genomics + metabolomics = basic biological mechanisms

Weaknesses

Limited number of metabolites

Data analysis: New approaches needed

Opportunities

Threats

Metabolomics: technology gap

External

Conclusions

Internal

Strengths

Close link to genomics

Genomics + metabolomics = basic biological mechanisms

Weaknesses

Limited number of metabolites

Data analysis: New approaches needed

Opportunities

Biochemistry

Behaviour genetics

New breeding opportunities for animal welfare

Threats

Metabolomics: technology gap

External

University of Bologna
Department of Agricultural and Food Sciences
Samuele Bovo
Gian Luca Mazzoni
Giuseppina Schiavo
Anisa Ribani
Valerio Joe Utzeri
Antonia Bianca Samoré
Emilio Scotti
Francesca Bertolini
Stefania Dall'Olio
Paolo Trevisi
Paolo Bosi

Department of Surgical and Medical Sciences
Endocrinology Unit
Flaminia Fanelli
Marco Mezzullo
Uberto Pagotto

Department of Statistical Sciences
Giuliano Galimberti
Daniela Giovanna Calò

Associazione Nazionale Allevatori Suini
associazione nazionale allevatori suini

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Innovagen project (MiPAAF)
AGER project (Fondazioni Bancarie)
FARB project (University of Bologna)

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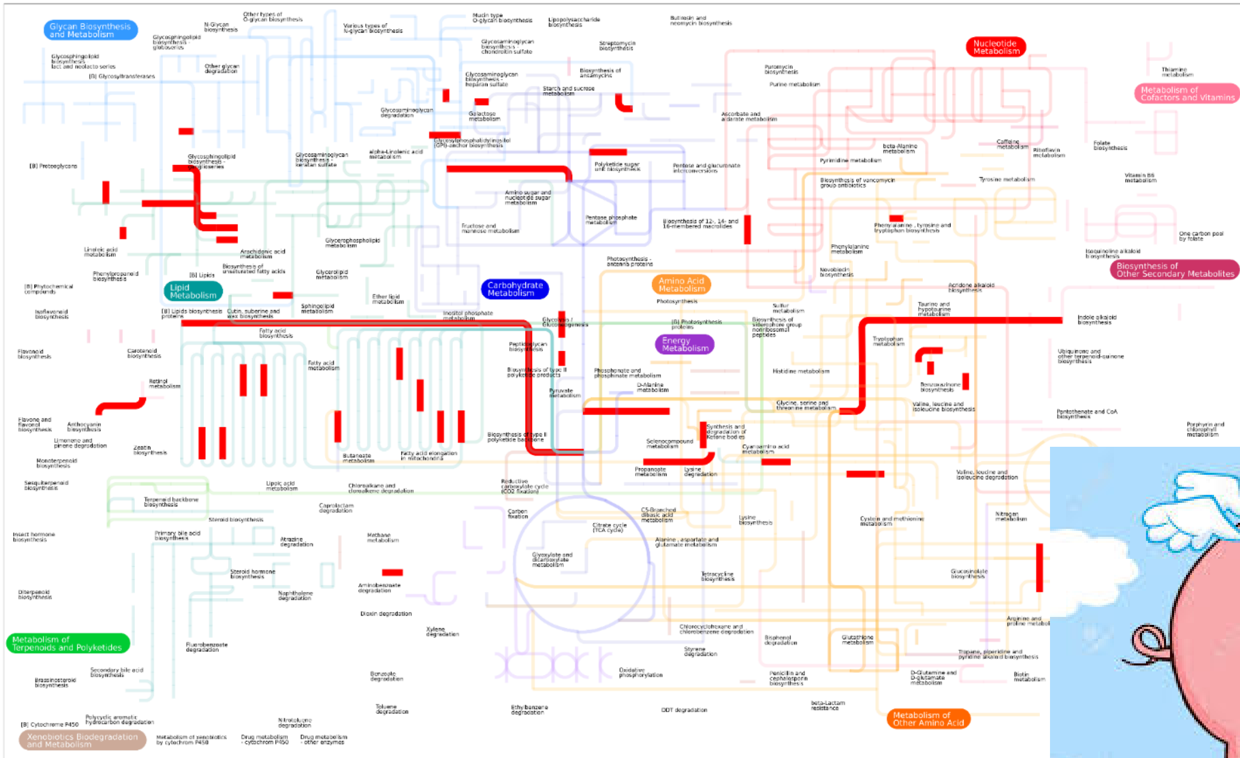
Glycan Biosynthesis and Metabolism
Nucleotide Metabolism
Metabolism of Cofactors and Vitamins
Biosynthesis of Other Secondary Metabolites
Lipid Metabolism
Carbohydrate Metabolism
Amino Acid Metabolism
Energy Metabolism
Metabolism of Terpenoids and Polyketides
Xenobiotics Biodegradation and Metabolism
Metabolism of Other Amino Acid

Thank you!

The added value of molecular phenotypes: towards the identification of animal welfare proxies

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