Exploiting the network-based association weight matrix approach for the genetic dissection of milk nitrogen fractions in dairy cattle

S. Pegolo[1]*, N. Mach[2], Y. Ramayo-Caldas[2,3], A. Rossoni[4], E. Santus[4], G. Bittante[1] & A. Cecchinato[1]

[1] *Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell'Università 16, 35020 Legnaro, Padua, Italy*
[2] *UMR 1313, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France*
[3] *Animal Breeding and Genetics Program, Institute for Research and Technology in Food and Agriculture (IRTA), Torre Marimon, Caldes de Montbui, 08140, Spain*
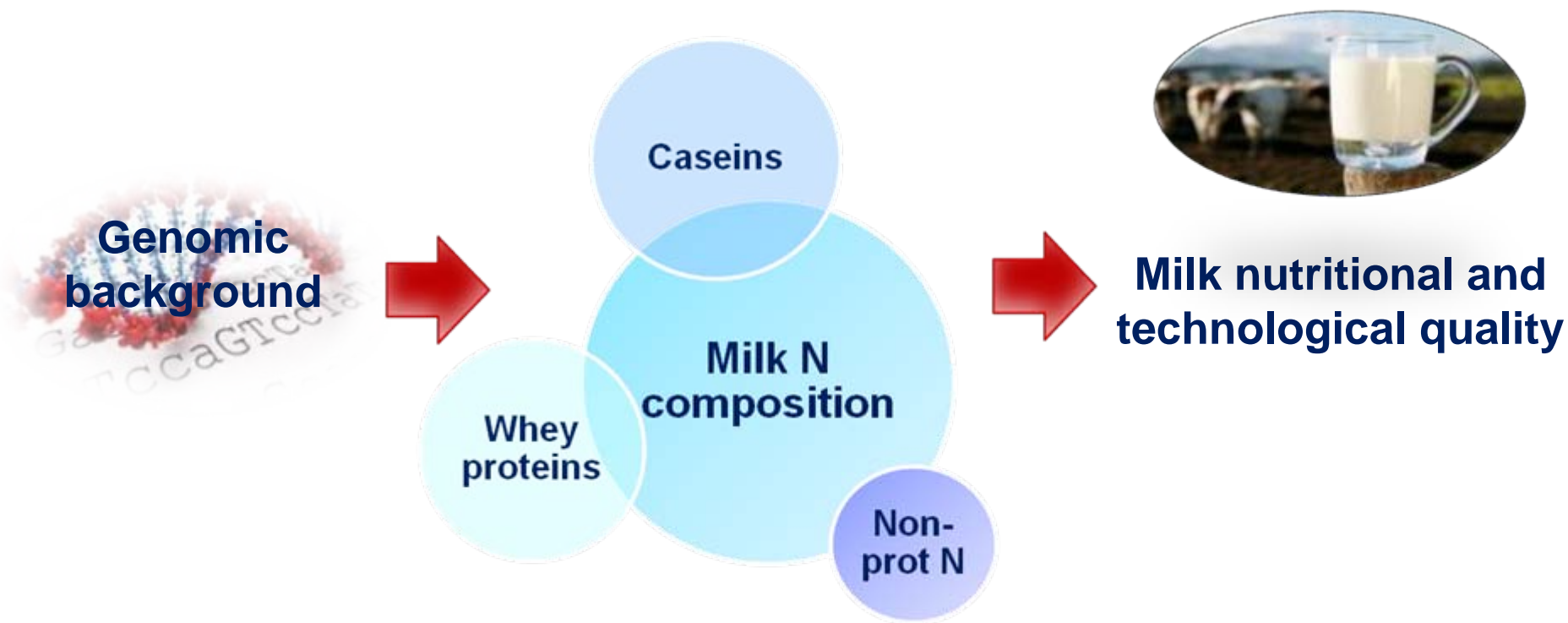[4] *Italian Brown Breeders Association, Loc. Ferlina 204, Bussolengo 37012, Italy*

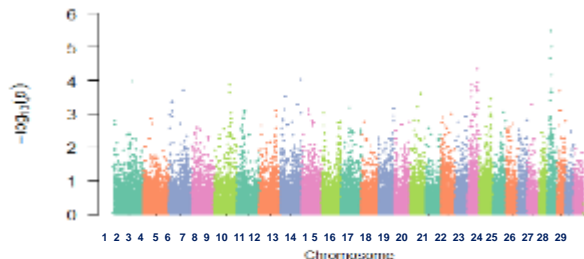*Dr. Enrico Santus*

*Corresponding author: sara.pegolo@unipd.it

Background

**Genomic background** → Caseins / Milk N composition / Whey proteins / Non-prot N → **Milk nutritional and technological quality**

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Aim of the study

GWAS results (P-val & additive effect)

Gene-set enrichment analysis

Associated weight matrix (AWM) and network framework

	κ-CN	β-LG	MUN
SUSD1	-0.925	-2.066	…
SSPN	1.177	-1.108	…
TERT	0.118	0.159	…
...	…	…	…
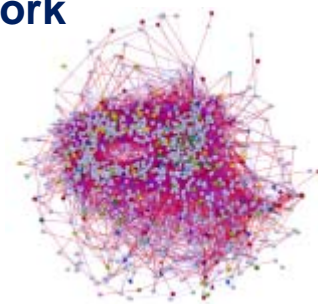
Deeper knowledge about genetic regulation of the physiological and cellular mechanisms required for milk protein synthesis and secretion

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Methods

GWAS
Single marker regression
(DIM, parity, herd as fixed factors, $P < 5*10^{-5}$
(*Genabel R package*)

Pathway analysis
SNP selection: SNP-to-gene distance <15 kb, $P < 0.05$ (*BiomaRt R package*)
Gene-set enrichment GO and KEGG databases, FDR<0.05 (*goseq R package*)

Gene-network analysis

- ❖ **Milk samples** from **1,011 Brown Swiss cows** (Cowability project)
- ❖ **37,568 SNPs** (Illumina Bovine SNP50)
- ❖ **Milk N composition** (caseins, whey proteins, non-prot N)

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Methods - AWM construction

SNP selection criteria from GWAS:

- ✓ **Selection of key phenotype** (κ-CN)

- ✓ **Primary SNP selection:** *P*≤0.05 for κ-CN

- ✓ **Secondary SNP selection:** SNPs with *P* ≤0.05 in ≥3 non- key phenotypes

- ✓ **SNP-to-gene distance:** <10 kb

- ✓ **One SNP-One Gene:** 1) >n° phenotypes; 2) lowest *P*-val

Pearson's r

	κ-CN	β-LG	…MUN
SUSD1	-0.925	-2.066	…
SSPN	1.177	-1.108	…
TERT	0.118	0.159	…
…	…	…	…

- ❖ **Rows:** 1,917 SNP/genes
- ❖ **Columns:** milk N composition
- ❖ **Cell:** normalized (z-scores) additive effect

PCIT algorithm
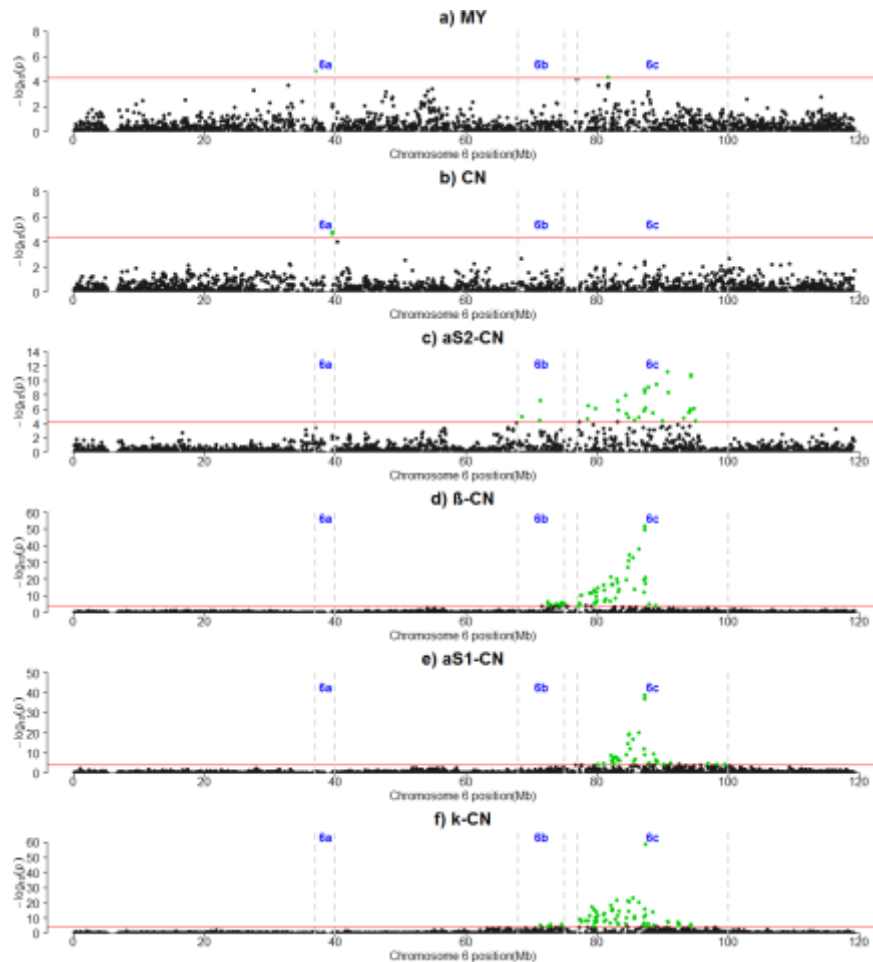**(Partial correlations - Information Theory)**

$r ≥|0.80|$

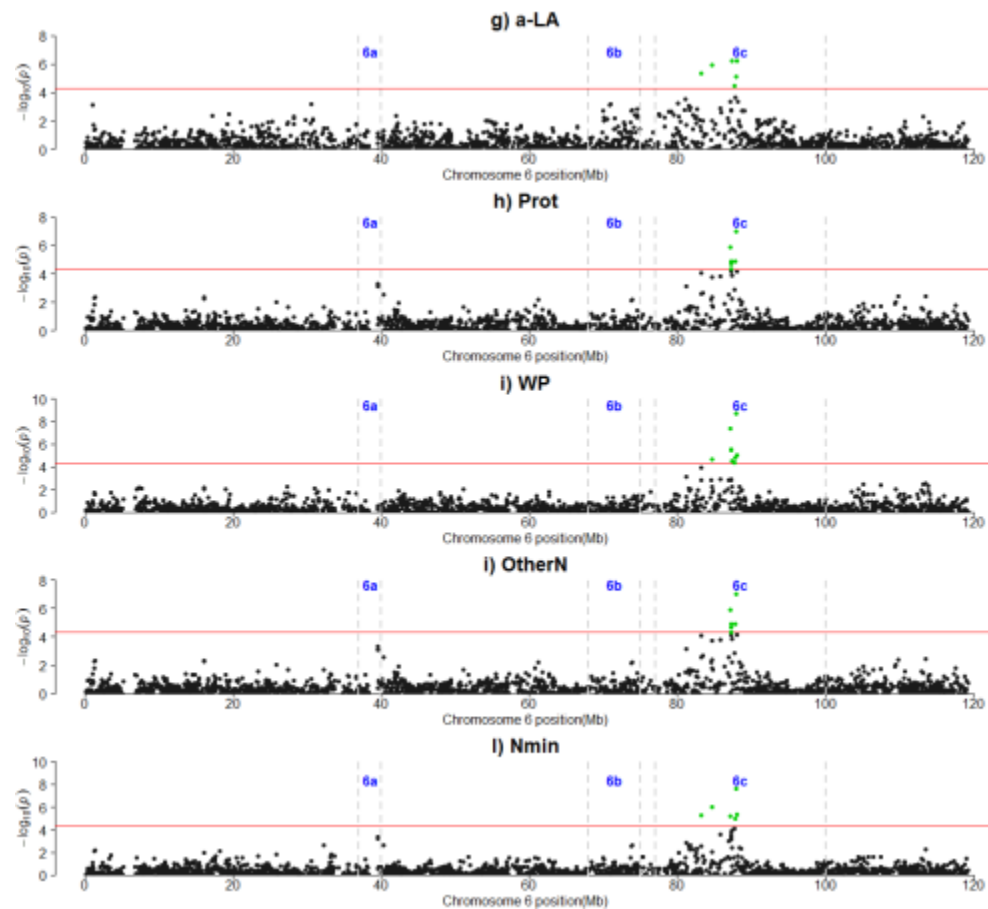## Biologically relevant interactions and key regulators
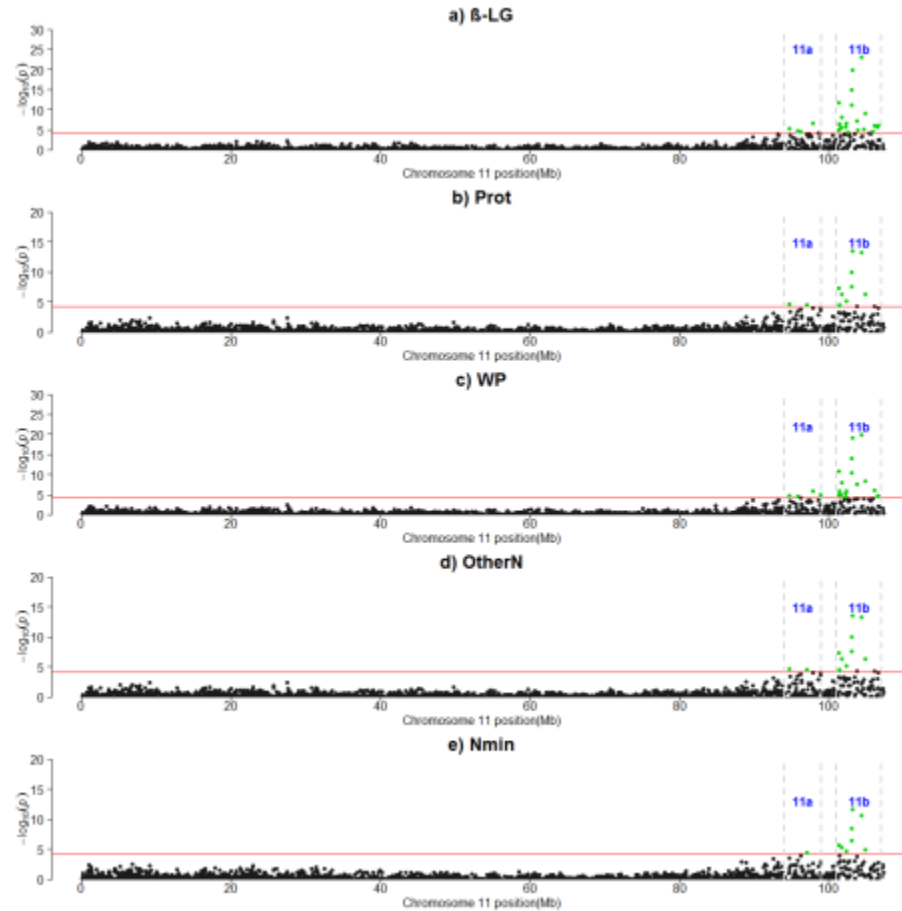*(Cluego, NetworkAnalyzer, IPA)*

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BTA6

a) MY

b) CN

c) aS2-CN

d) ß-CN

e) aS1-CN

f) k-CN

BTA6

BTA11

Results – GWAS

GWAS → **170 SNPs:** **103** SNPs on **BTA6** (~77.19-99.45 Mbp, including casein cluster); **22** SNPs on **BTA11** (~101.27 - 106.54 Mbp, including the *PAEP* gene)

Heritability estimations are in general high
Heritability for casein fractions are higher than total casein

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Results – GWAS and pathway analysis

PATHWAY ANALYSIS
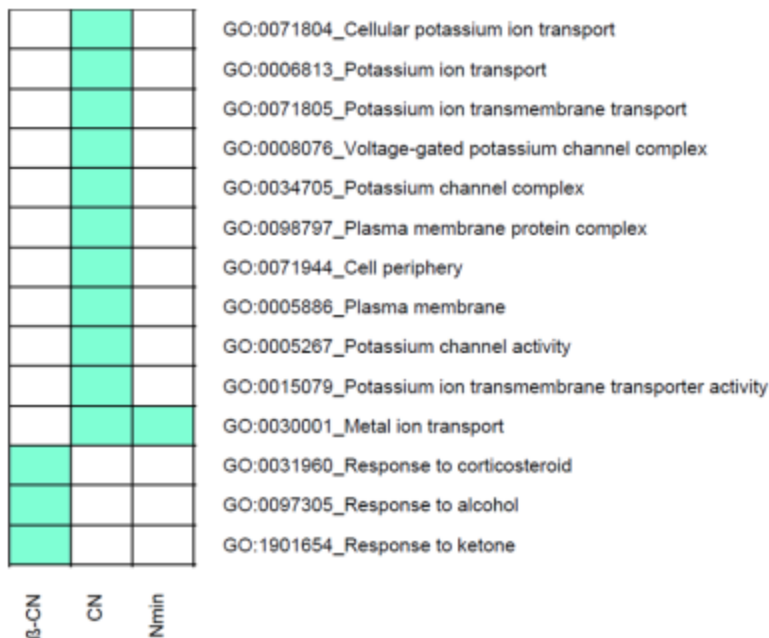
37000 SNPs

17000 SNPs btw 15Kb (flanking)

13000 genes

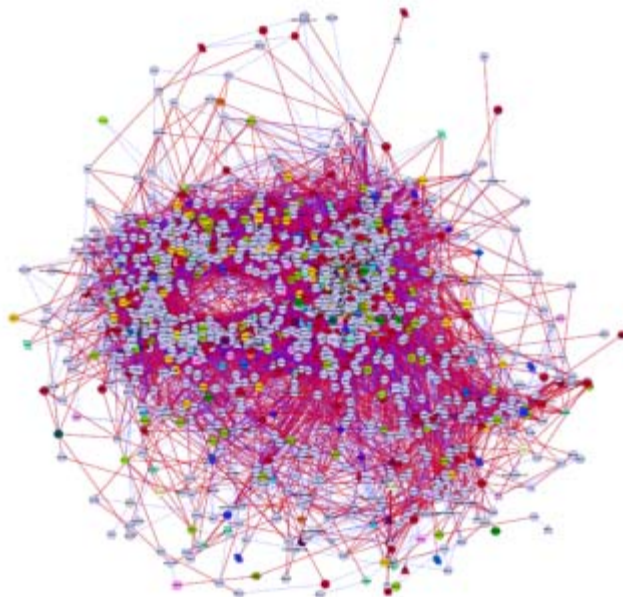600 genes with significant associations with MY or PROT

Results – GWAS and pathway analysis
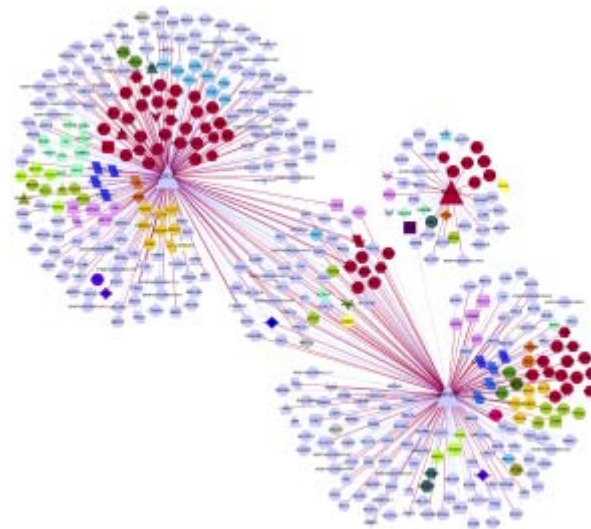
PATHWAY ANALYSIS
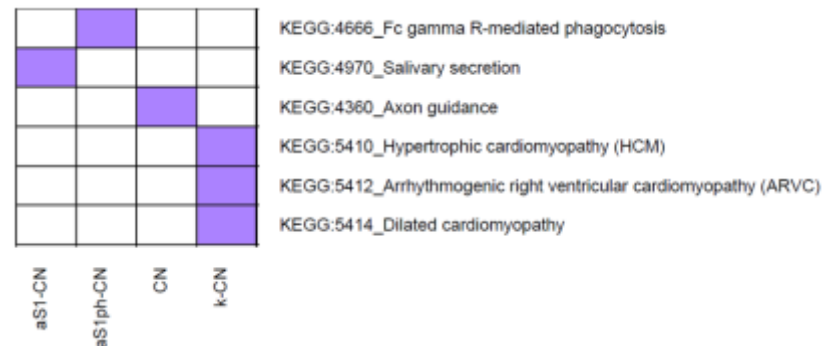
Results – *Network analyses*

1,904 nodes/genes
101,284 edges/interactions
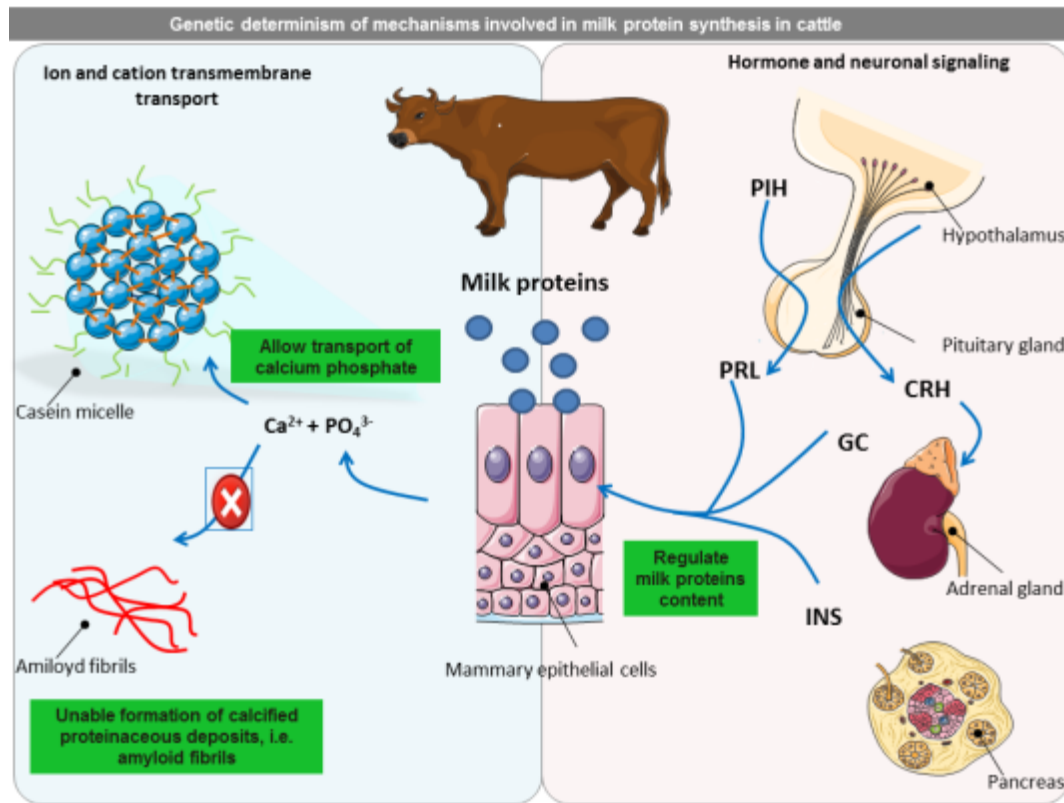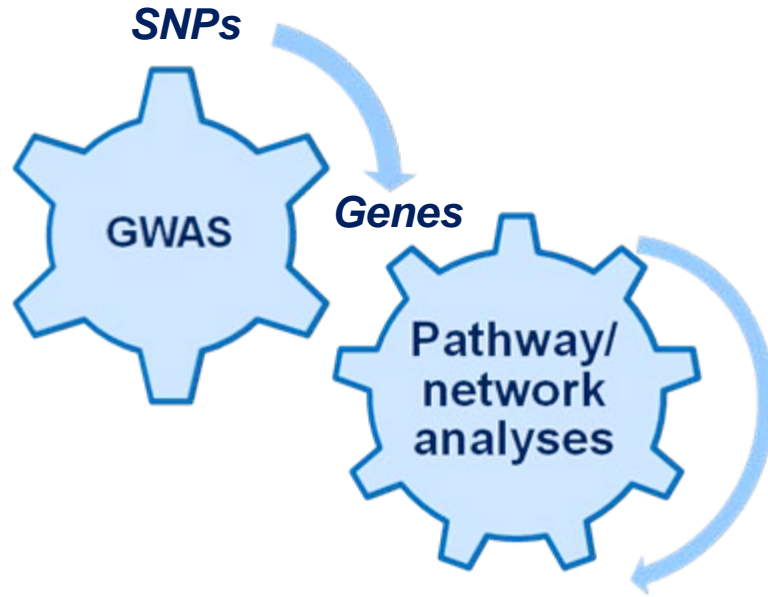
TF-trio
GFI1B*, *NR5A1* and *ZNF407

452 genes (24% of genes in the filtered AWM)

Results - Functional analyses

Conclusions

SNPs

GWAS

Genes

Pathway/ network analyses

✓ **Genetic control of milk protein composition**

✓ **Mammary gland functionality**

✓ *GFI1B, NR5A1 and ZNF407 as* **key regulators**

Validation studies?

FUTURE

Selection strategies to improve milk quality and technological characteristics

Thank you for your attention!