

# Validation of candidate causative variants on milk composition and cheese-making properties in Montbéliarde cows

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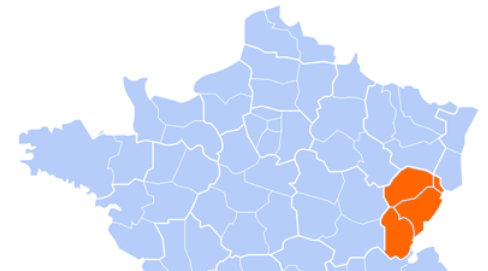
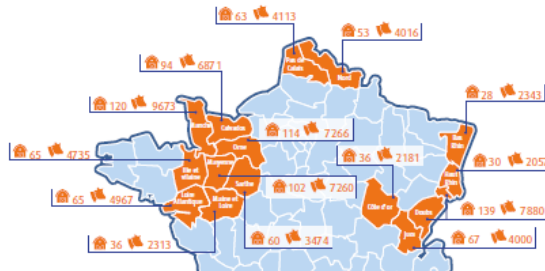
# Background

Milk **cheese-making properties** (CMP) strongly related to **milk composition**

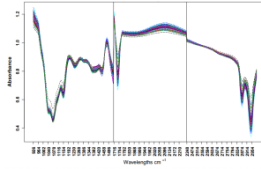
Milk CMP & composition can be predicted by mid-infrared (**MIR**) spectrometry

**PhenoFinlait** & **FROM'MIR** projects to predict these traits at a large-scale to identify **candidate causative mutations**

PhenoFinlait



# The PhénoFinlait project



Milk protein & fatty acid composition

**GWAS with  $\approx 9,000$  cows on imputed WGS**



Identification of the best candidate causative variants in 24 candidate genes (Sanchez et al., 2017; Boichard et al., 2016)

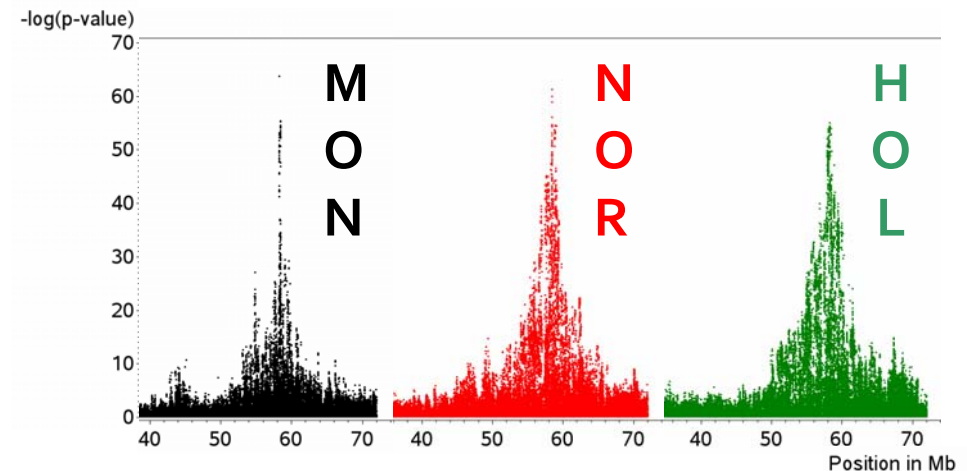
Design on the **custom part** (v6 & v7) of the **EuroG10K** Beadchip (see Boichard et al. Friday 1:30)

Montbéliarde Normande

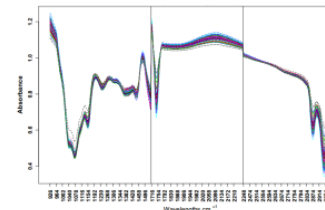
Holstein



Ex: BTA20  $\alpha$ -lactalbumin

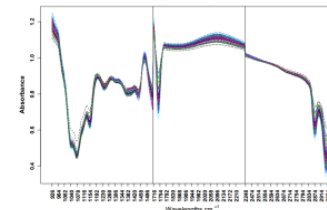


# FROM'MIR - Phenotypes



	Traits	# Traits	Equations	R <sup>2</sup>
<b>Milk CMP</b> Equations of prediction from <b>420 milk samples</b> with CMP reference analyses & MIR spectra	Cheese Yields	3	FROM'MIR	0.54 - 0.89
	Coagulation	9	FROM'MIR	0.43 - 0.76
	Acidification	3	FROM'MIR	0.39 - 0.62

# FROM'MIR - Phenotypes

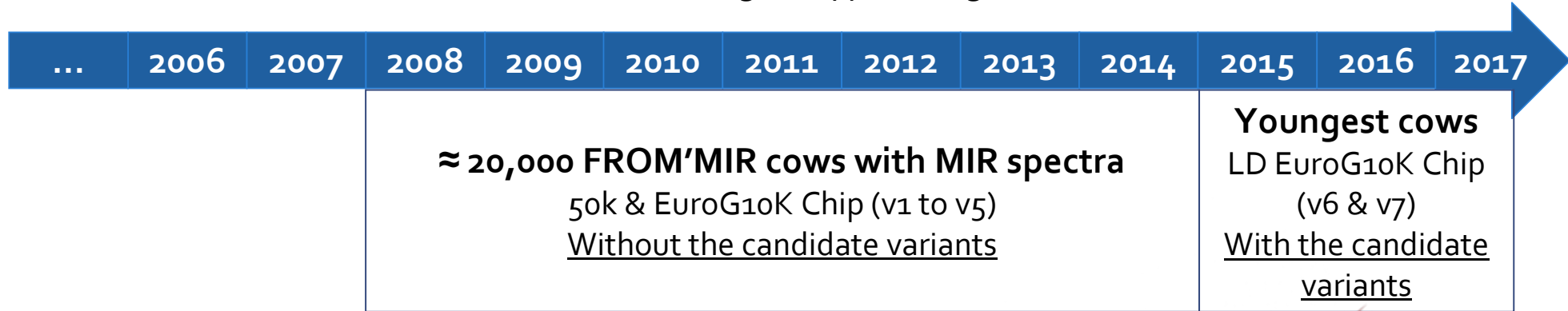


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	Acidification	3	FROM'MIR	0.39 - 0.62
<b>Milk composition</b> Equations of prediction from previous projects	Proteins	6	PhenoFinlait	0.59 - 0.92
	Fatty acids	4	PhenoFinlait	0.76 - 1
	Minerals	5	Optimir	0.68 - 0.92

Application of equations on  
 ≈ **6 millions of MIR spectra** from **330,000 Montbéliarde cows** (Eastern France)

# FROM'MIR – genotypes, imputations & GWAS

Year of birth of  $\approx 180,000$  Montbéliarde cows genotyped for genomic selection (50k and EuroG10k Chips)



1

« **Backward** » imputations with FImpute  
to impute 50k + variants of the custom part  
from the most recent and the most complete  
versions of the EuroG10k Chip (v6 & v7)  
for FROM'MIR cows

2

**GWAS** with GCTA  
Effects of the 45,120 SNP with  $MAF \geq 1\%$   
+ Random polygenic effects of animals  
(GRM 50k SNP)

# Results

## « Backward » imputation accuracies (variants with $MAF \geq 1\%$ )

Mean squared correlation between imputed / true genotypes in a validation set  $\approx 92\%$

## GWAS significant results ( $-\log(P\text{-value}) > 6$ )

1069 variants with significant effects on at least 1 of the 30 traits

Most of them located in regions previously identified for milk composition in the PhénoFinlait project

	QTL	Candidate genes	« Custom » variants in candidate genes
« Custom » variants <b>identified</b> in <b>PhénoFinlait</b> cows	<b>13</b>	<b>24</b>	<b>245</b>
« Custom » variants <b>validated</b> in <b>FROM'MIR</b> cows	<b>13</b>	<b>18</b>	<b>167</b>

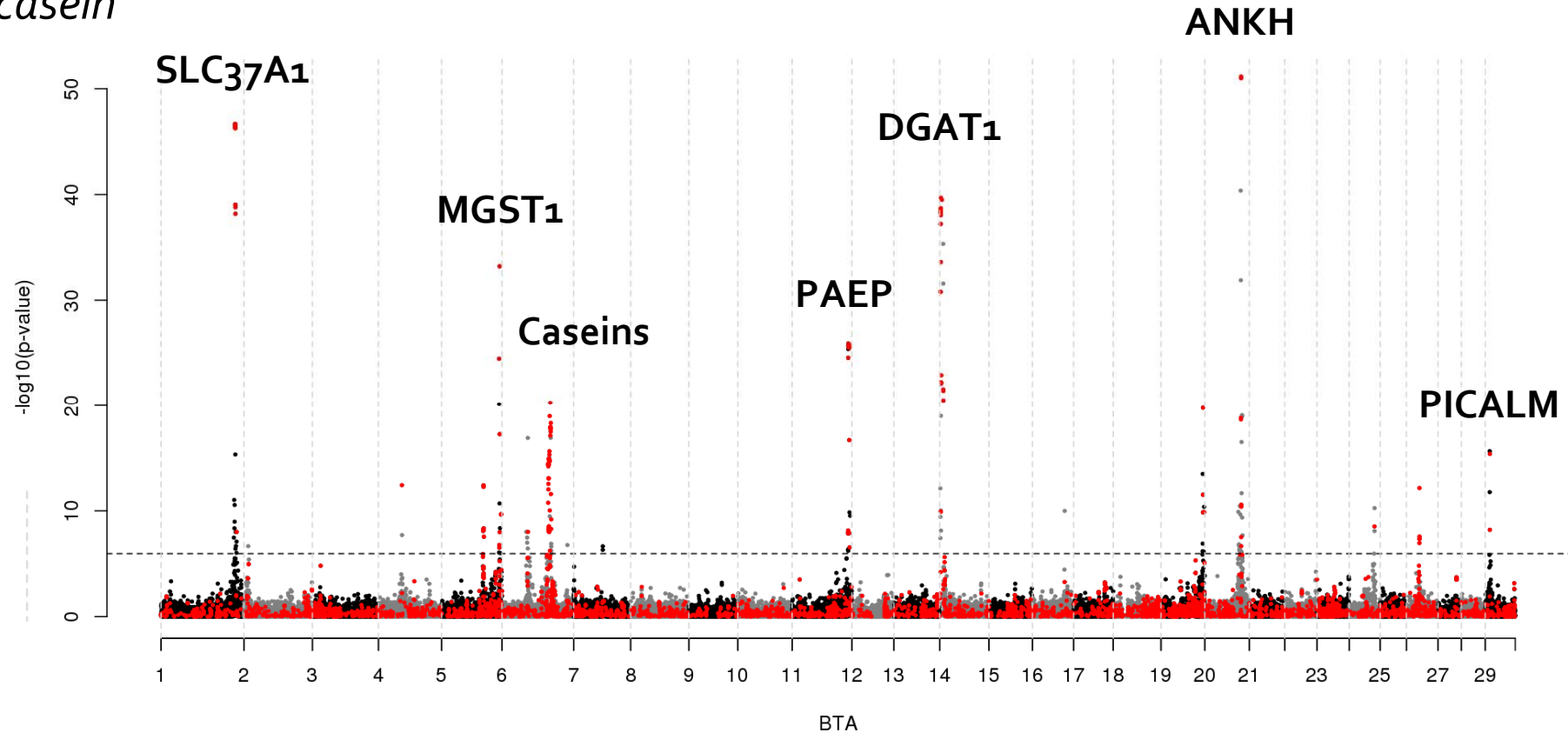
Including **115** variants in TOP10



# Results

FROM'MIR Montbéliarde cows: validation of QTL for milk **protein composition**

*Ex.  $\alpha$ s1-casein*

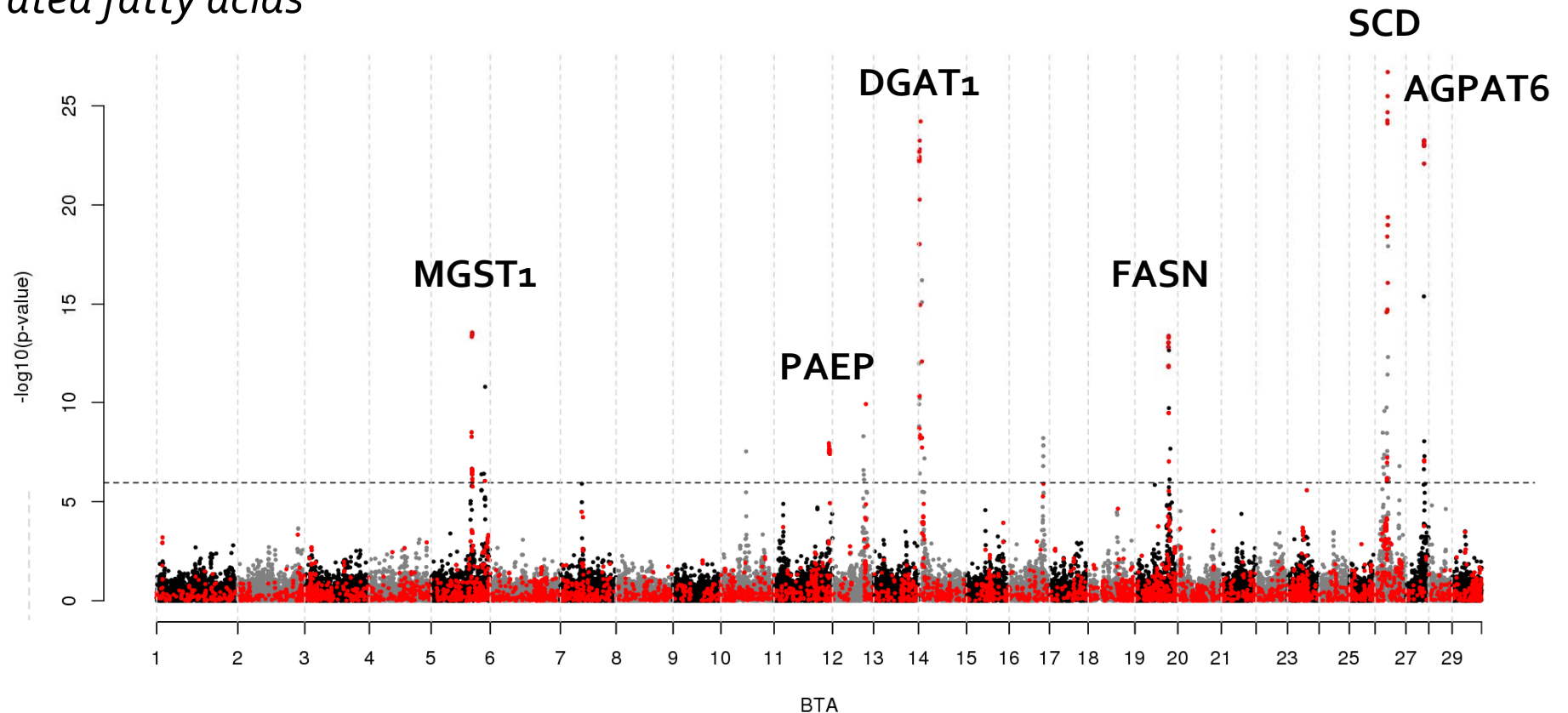




# Results

FROM'MIR Montbéliarde cows: validation of QTL for milk **fatty acid composition**

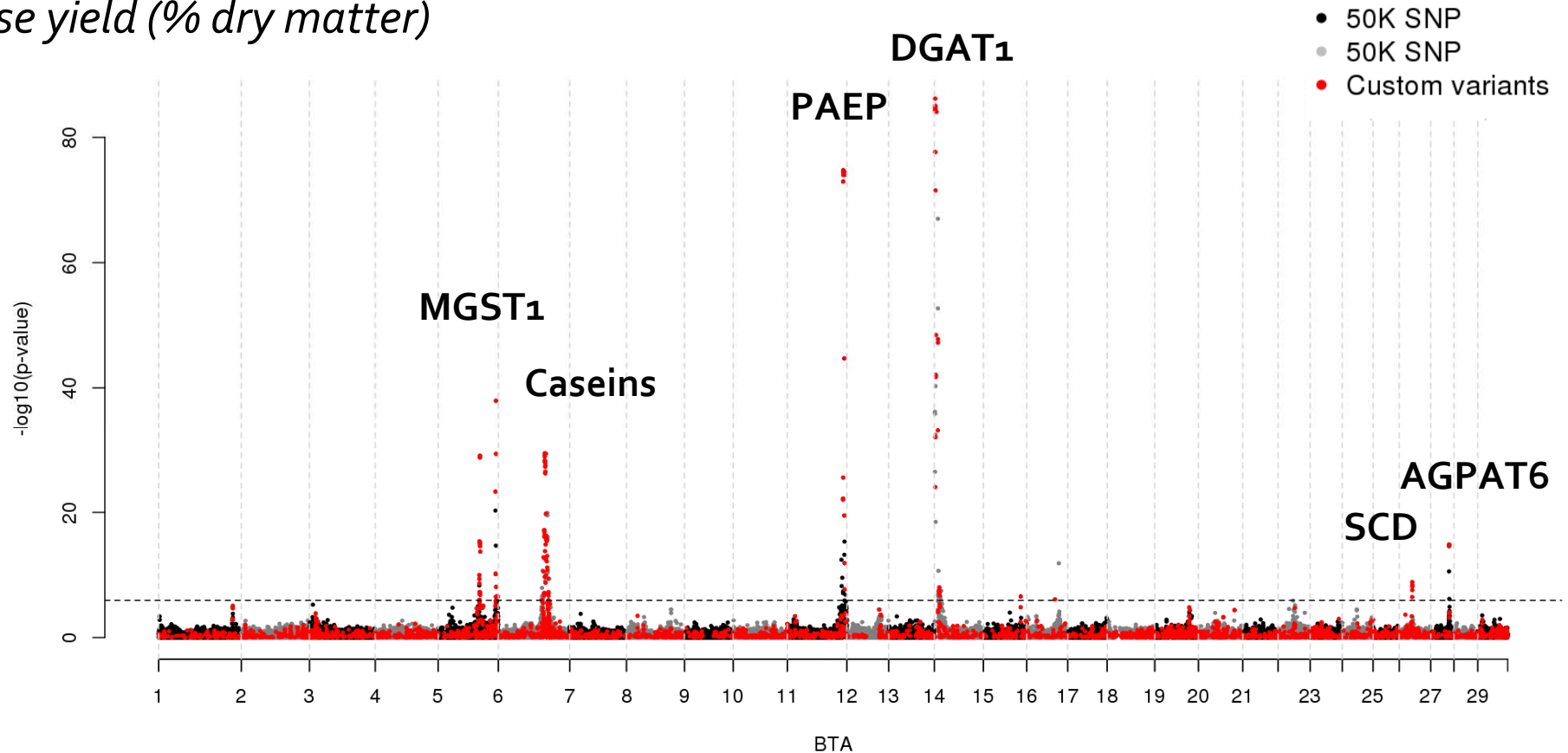
*Ex. saturated fatty acids*



# Results

FROM'MIR Montbéliarde cows: validation of QTL for milk **CMP** traits

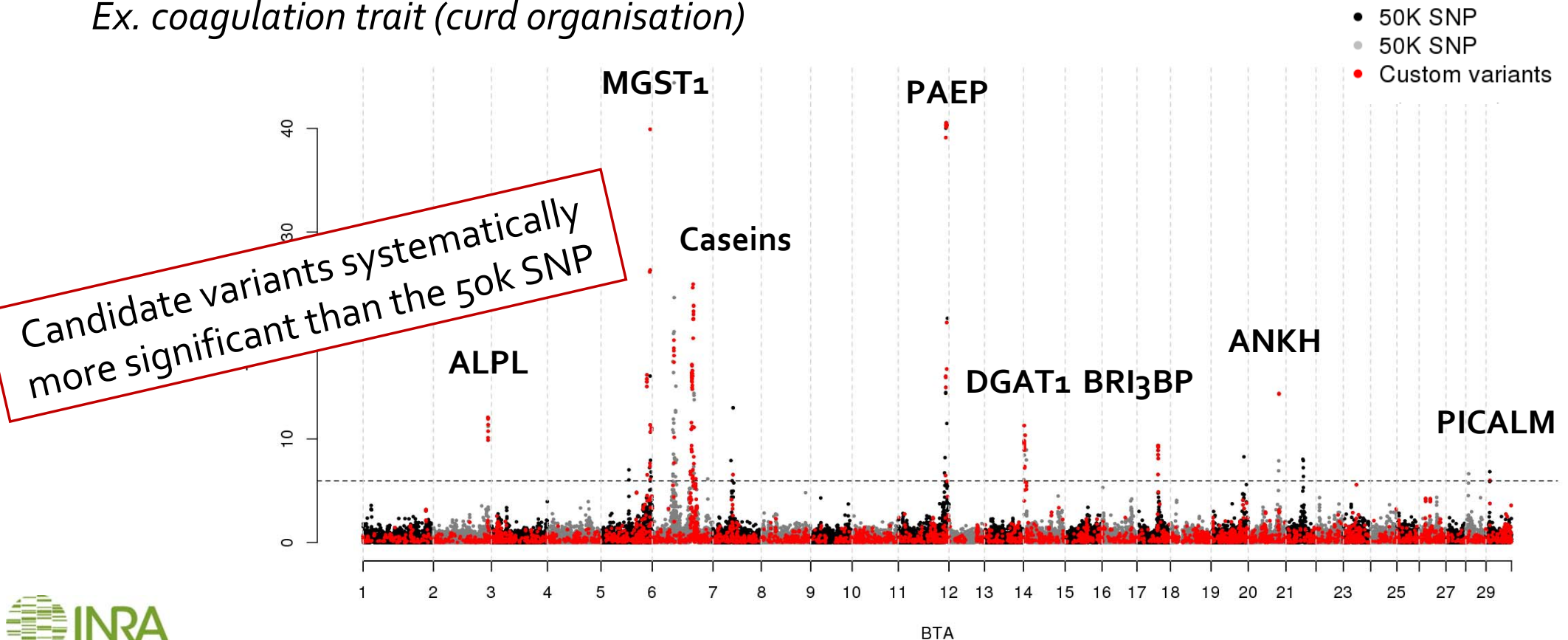
*Ex. cheese yield (% dry matter)*



# Results

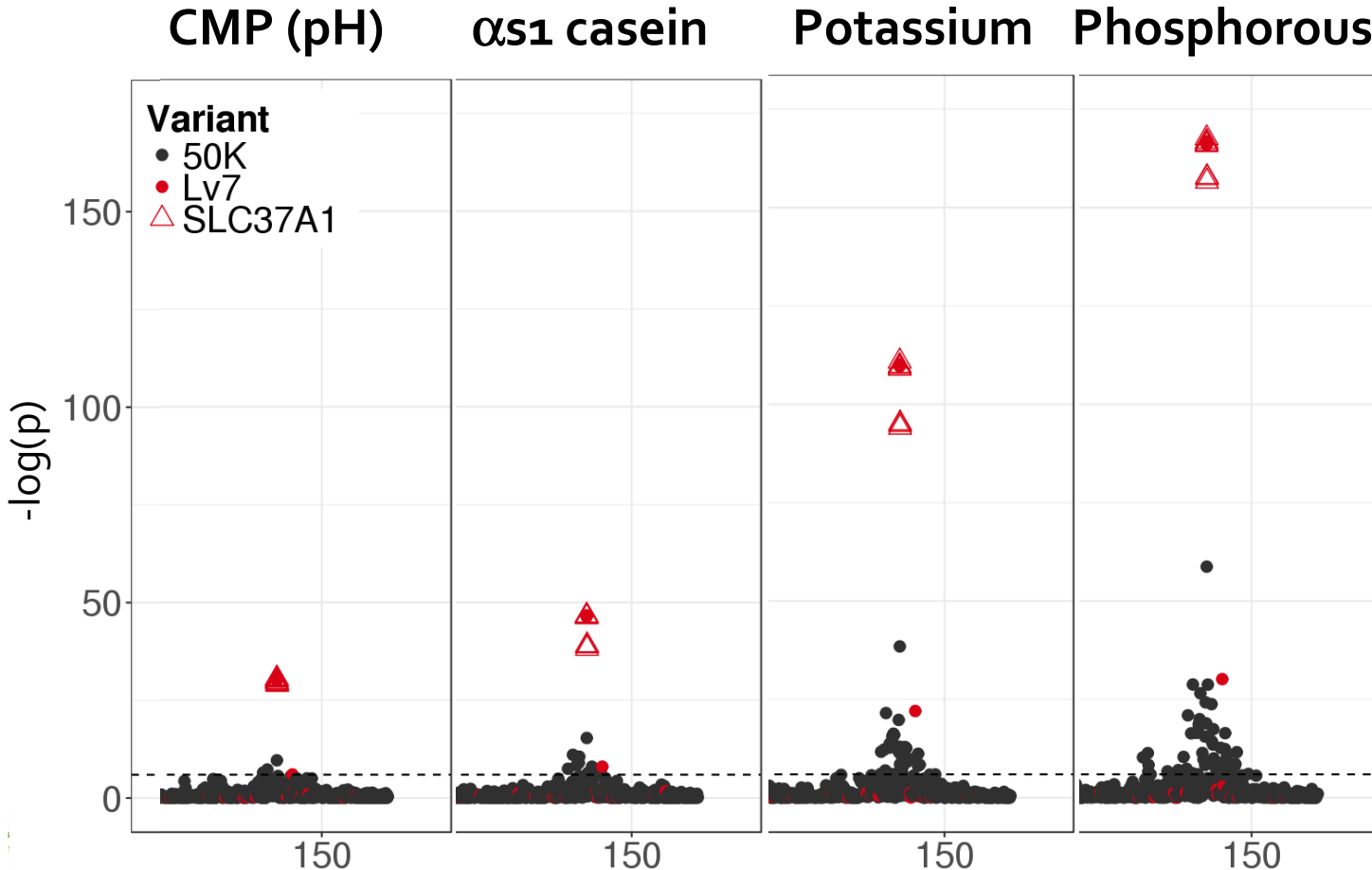
FROM'MIR Montbéliarde cows: validation of QTL for milk **CMP** traits

*Ex. coagulation trait (curd organisation)*



# Results

Ex. QTL on BTA1 at  $\approx 144,4$  Mb / **SLC37A1** (glucose-6-phosphate transporter)

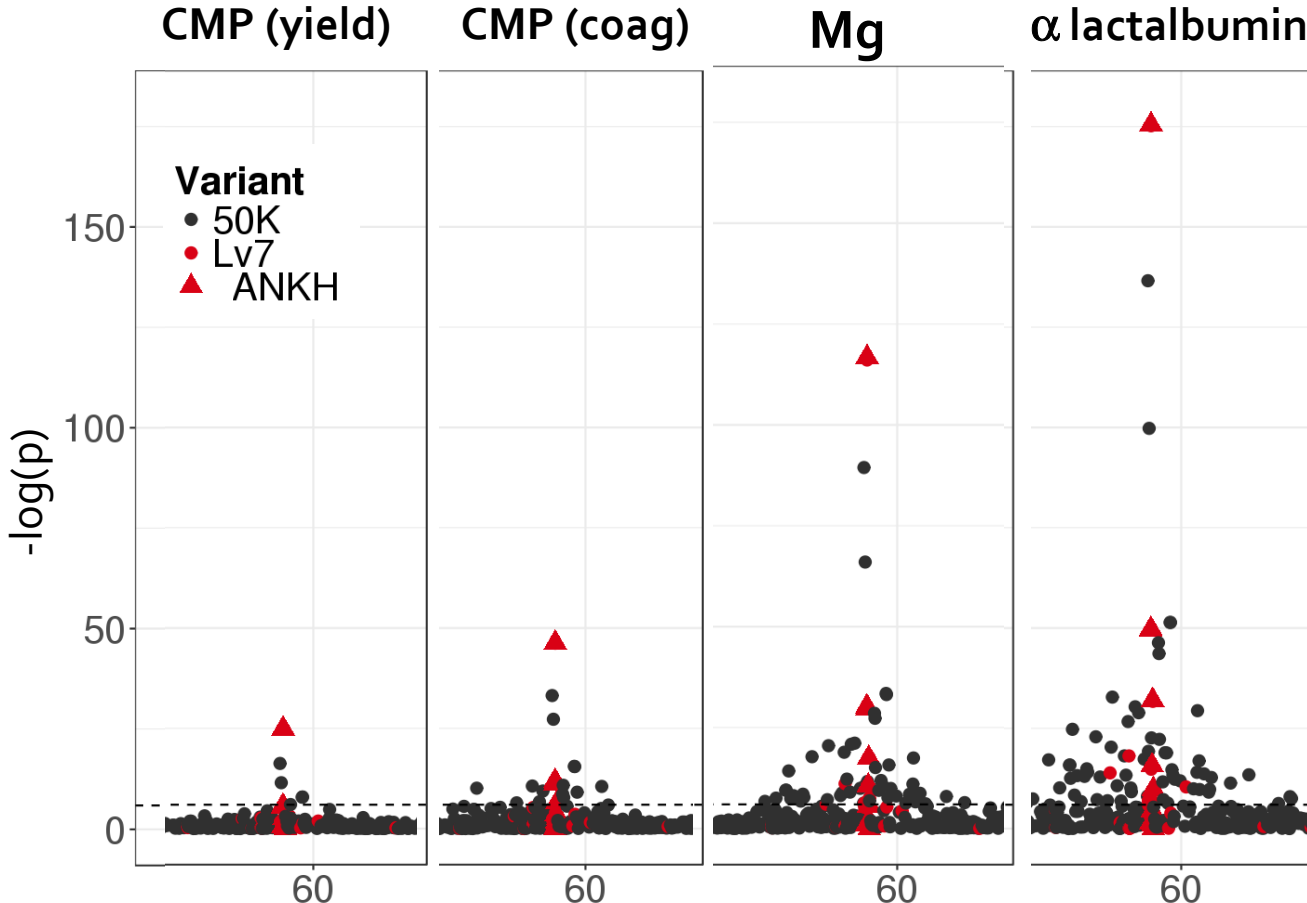


« Finer » phenotypes  
→ Higher peaks  
→ Better targetting of candidate mutations  
→ Functional link between complex trait and gene

The **best candidate** variant in intronic region of SLC37A1 at 144,398,764 bp

# Results

Ex. QTL on BTA20 at  $\approx 58,4$  Mb / **ANKH** (inorganic pyrophosphate transport regulator)



## Functional links

$\alpha$  lactalbumin = milk protein exhibiting a high affinity to Ca & ANKH encodes a transport regulator that helps to prevent the deposition of Ca in bones

The **best candidate** variant in intronic region of ANKH at 58,427,343 bp

# Results

The best candidate causative variant retained for each candidate gene

Functional annotations	Genes
5 Missense	ALPL - SLC26A4 - CSN3 - RECQL4 - SCD
5' UTR region	AGPAT6
3' UTR region	GPT
5 in upstream region	CSN1S1 – CSN1S2 – <b>PAEP*</b> – <b>DGAT1*</b> – PICALM
6 in introns	SLC37A1 – MGST1 – CSN2 – BRI3BP – FASN – ANKH

- \***DGAT1 & PAEP** genes => missense variants found as causal variants were not the most significant
- DGAT1**: K232A missense mutation also significant but with MAF < 1%
- PAEP**: missense mutations (103,303,475 & 103,304,757 bp) in strong LD with the TOP1 but not the most significant

# Discussion - Conclusion

Validation of candidate genes / variants detected for milk composition in 13 regions in an independent population of Montbéliarde cows for both CMP and composition

Analysing both traits of interest (complex) & fine-scale phenotypes can help to target the causative variants and to establish the functional link between these traits and candidate genes

We confirm effects of 18 candidate genes and propose the best candidate causative variant in each gene



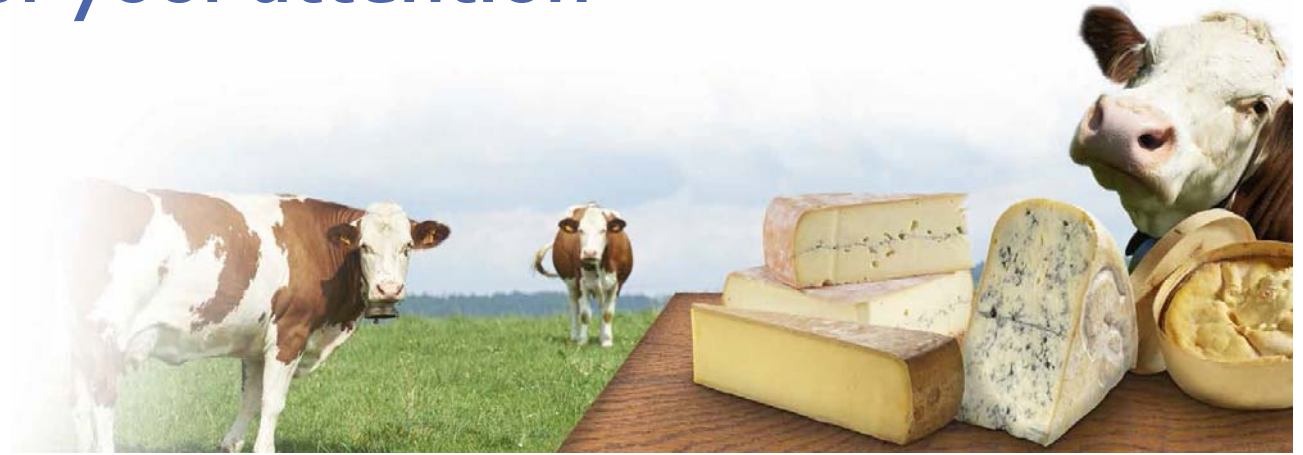
Identification of other candidate variants in FROM'MIR cows

- GWAS on imputed WGS (RUN6 of the 1000 bull genome project)
- Search for interacting genes co-associated with CMP and milk composition



# Thank you for your attention

!



Phénofinlait

ANR  
AGENCE  
NATIONALE  
DE LA  
RECHERCHE

