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**BovReg**  
Understanding cattle genomes

## ➤ Sequence-based GWAS meta-analyses for beef production traits

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**ELIANCE**  
Des éleveurs. Une ambition.

**Université de Limoges**

**Qualitas**

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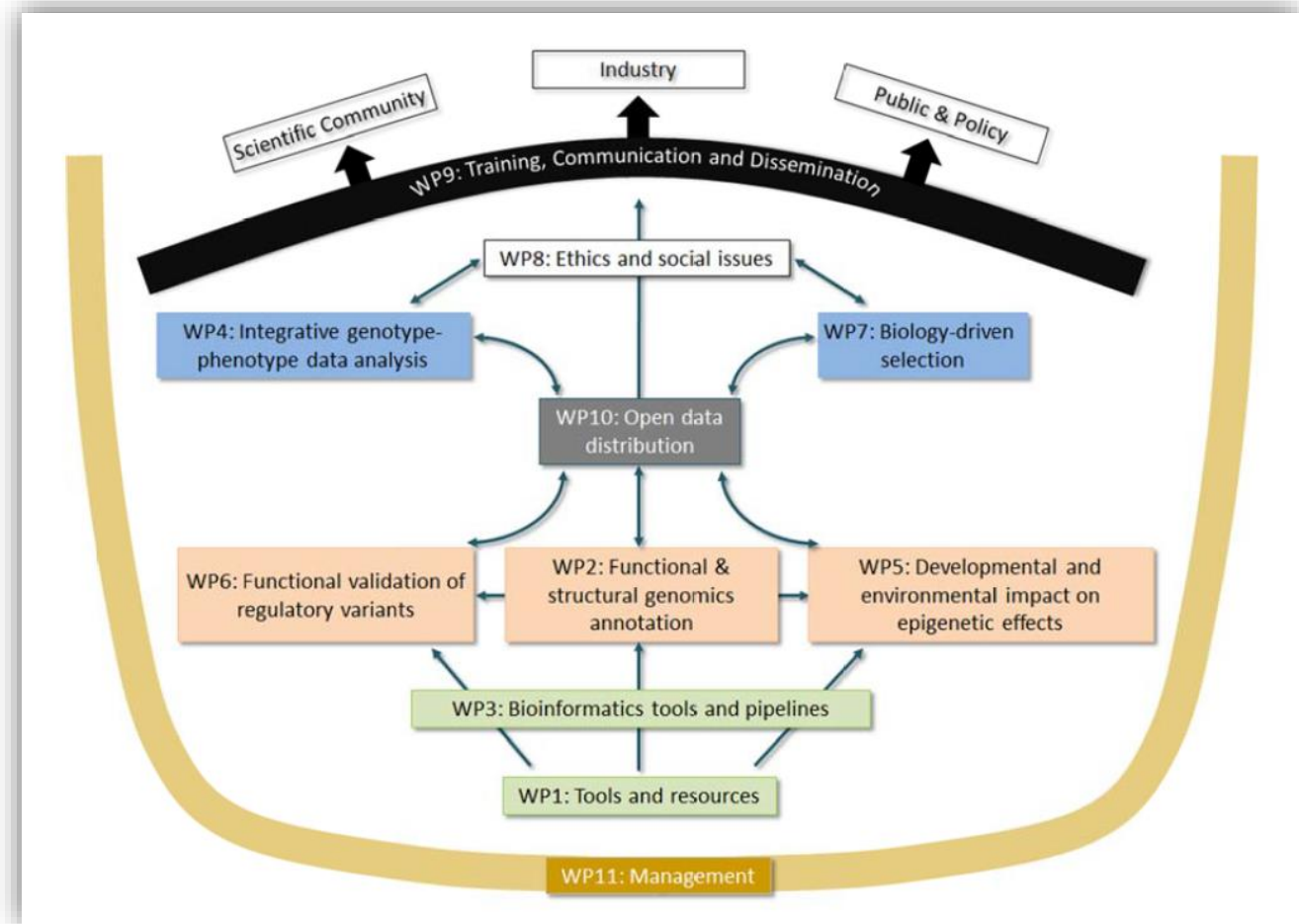


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# H2020 BovReg project

Identification of functionally active genomic features relevant to phenotypic diversity and plasticity in cattle

20 partners from 14 countries



11 WP incl. WP4

Integrative analysis of genotype-phenotype



# WP4 – Integrative genotype-phenotype data analysis

**WP leader:** Hubert PAUSCH (ETH, Switzerland) - 11 partners / 20 involved in BovReg

**T4.1 – Hubert PAUSCH (ETH, Switzerland)**  
**GWAS and meta-analyses from whole-genome sequences (WGS) for biological efficiency, disease resistance and fertility traits**

T4.2 – Carole CHARLIER (GIGA, Belgium)  
Phenotypic impact of mobile element integration

T4.3 – Christa KUHN (FBN, Germany)  
eQTLs and mQTLs analyses

T4.4 – Emily CLARK (UEDIN, UK)  
Tools to prioritize candidate causative variants










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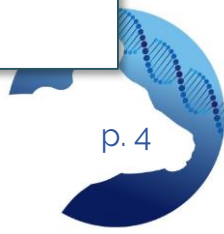
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# T4.1 – GWAS & meta-analyses

<p><b>Within-breed GWAS</b></p>				
<p><b>4 groups of traits</b></p>	<p><b>Mastitis resistance</b></p>	<p><b>Milk yield &amp; fertility</b></p>	<p><b>Feed efficiency</b></p>	<p><b>Beef traits</b></p>
<p><b>Meta-analyses</b></p>				
	<p>8-13 populations &gt; 120 000 anim.</p>	<p>7-12 populations &gt; 125 000 anim.</p>	<p>3-9 populations &gt; 13 000 anim.</p>	<p>3-10 populations &gt; 25 000 anim.</p>
<p>Bulls, cows, steers from purebred or crossbred populations Phenotypes (with weights): YD, DYD, DRP, AP</p>				



# T4.1 – Within-breed GWAS & meta-analyses

All partners applied similar imputation and GWAS workflows before meta-analyses



## Imputations

2 steps

50k → 777k (HD)  
HD → WGS

Within-breed animals with HD genotypes  
Animals of various breeds with WGS (1000 BG project)

*Fimpute / Minimac / Beagle*



**tens of millions of variants  
incl. causal variants**



## GWAS

*GCTA*

Linear mixed model to test individual variant effect together with a polygenic effect estimated from a GRM built using 50k genotypes (when required, phenotypes were weighted)



Meta-analyses

*QC + METAL*



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# T4.1 – « Beef » MA – populations

## A large number and a large diversity of populations

- ☒ 8 purebred populations from 5 French breeds (NOR, MON, CHA, LIM, BLA)



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- ☒ 4 populations from Swiss breeds (BSW, OBR)



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- ☒ 2 crossbred populations from Germany (HOL x CHA)



- ☒ 1 composite line from Canada (ANG, CHA, beef)



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# T4.1 – « Beef » MA – traits analyzed

**A large number and a large diversity of traits**

- Growth (6)
- Morphology (6)
- Carcass (21)

1	Growth	Birth Weight	BW
2	Growth	weight at month 15	W15
3	Growth	weight at 18 months	W18
4	Growth	weight at 24 months	W24
5	Growth	average daily gain	ADG
6	Growth	average daily gain during fattening	ADG
7	Morphology	muscularity score	MS30
8	Morphology	skeletal score	SS30
9	Morphology	thickness of bones	TB30
10	Morphology	Thighs	THIGHS
11	Morphology	Wither	WITHER
12	Morphology	Fat score	FS
13	Carcass	carcass weight	CW
14	Carcass	fat coverage	CF
15	Carcass	meatiness	MT
16	Carcass	Area of longissimus thoracis	ALT
17	Carcass	Carcass conformation	CC
18	Carcass	carcass fat score	FS
19	Carcass	carcass yield	CY
20	Carcass	Internal fat weight	IFW
21	Carcass	length of the leg	LL
22	Carcass	Rib Eye Area	REA
23	Carcass	Weight at slaughter	WS
24	Carcass	Maximum width of the thigh	WT
25	Carcass	age at slaughter	AS
26	Carcass	carcass grade	CG
27	Carcass	average backfat thickness	ABT
28	Carcass	hot carcass weight	CW
29	Carcass	lean meat yield	LMY
30	Carcass	fat content of 6th rib	FC6
31	Carcass	fat content measured by ultrasound	FCU
32	Carcass	muscular development	MD
33	Carcass	skeletal development	SD

## Grouping of traits in 16 MA

MA	Trait type	Traits	# traits	# pop.	# partners	# anim.
1	Growth	W15/W18/ADG	3	7	4	18774
2	Growth	BW	1	5	2	2720
3	Morphology	MS30/THIGHS/CC	3	6	2	17418
4	Morphology	MS30/WITHER/CC	3	6	2	17418
5	Morphology	LL	1	5	2	3695
6	Morphology	WT	1	5	2	3695
7	Morphology	SS30/SD	2	4	2	12140
8	Carcass	CW	1	7	4	19989
9	Carcass	AS	1	6	2	12208
10	Carcass	CY	1	5	2	3694
11	Carcass	CG/LMY/MT/CC	4	10	5	25367
12	Carcass	FS/ABT/FC6/FCU/CF	5	8	5	14622
13	Carcass	WS	1	5	2	2636
14	Carcass	ALT	1	5	2	3692
15	Carcass	IFW	1	5	2	3686
16	Carcass	REA	1	3	2	4453

- 1 to 5 traits / MA
- 3 to 10 populations / MA
- 2 to 5 partners / MA
- 2600 to 20,000 animals / MA



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# T4.1 – « Beef » MA – methods

## 2 methods used

METAL software (Willer et al., 2010)

### z-score

For each variant, Z is calculated by combining the p-value ( $p_i$ ) associated to its effects in the different GWAS, weighted by the sample size ( $w_i$ )

$$Z = \frac{\sum_i Z_i w_i}{\sqrt{\sum_i w_i^2}}$$
$$Z_i = \Phi^{-1}\left(1 - \frac{P_i}{2}\right) \text{ (effect direction for study } i\text{)}$$

### Fixed effects

Normalized effect of each variant estimated in the GWAS  $i$  ( $\theta_i$ ) combined and weighted by the inverse of the error variance ( $w_i$ )

$$\hat{\theta}_F = \frac{\sum_i w_i \hat{\theta}_i}{\sum_i w_i}$$

➔ The fixed effects method is generally more powerful but as variant effects are considered as identical between GWAS, traits analyzed in GWAS need to be identical and measured in the same unit  
=> standardization of the effects by the genetic SD of the trait specific to each population

Effect of a variant was considered significant if  $-\log_{10}(\text{p-value}) \geq 8.7$   
=> 5% threshold after Bonferroni correction (~25M variants)



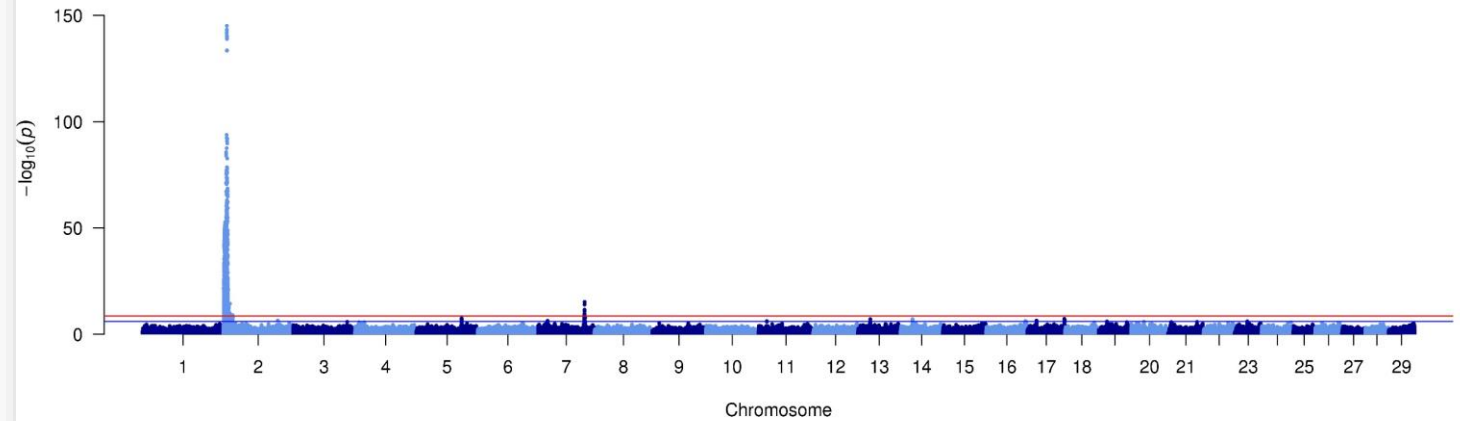
# T4.1 – « Beef » MA – results

Significant results (QTL) for 15 of the 16 MA on 11 bovine autosomes

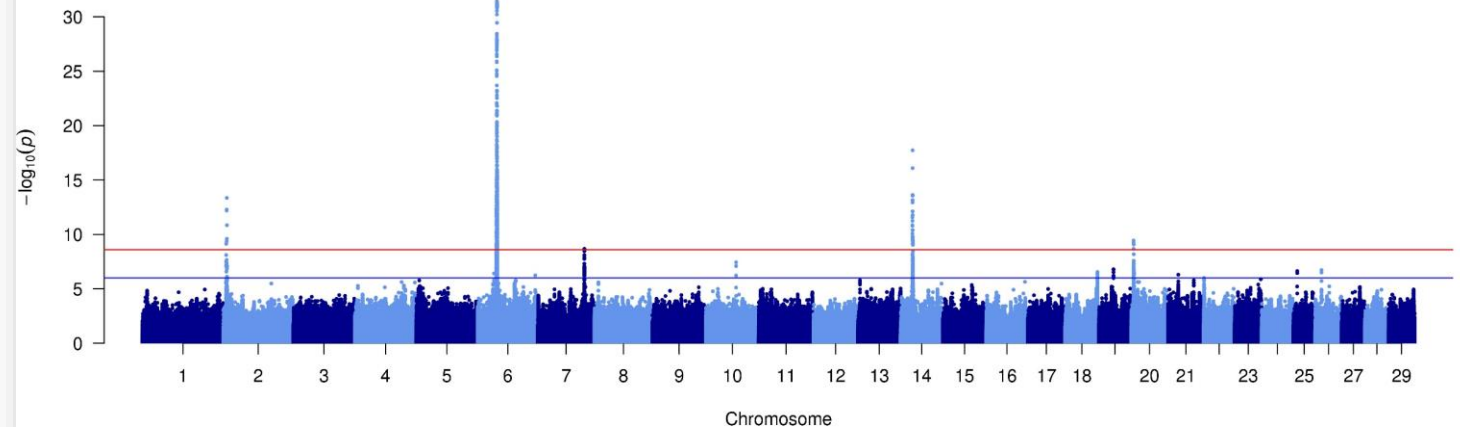
The most significant QTL located

On chromosome 2

Morphology MA2 (Fixed Effects method)



Carcass MA8 (Fixed Effects method)



On chromosome 6

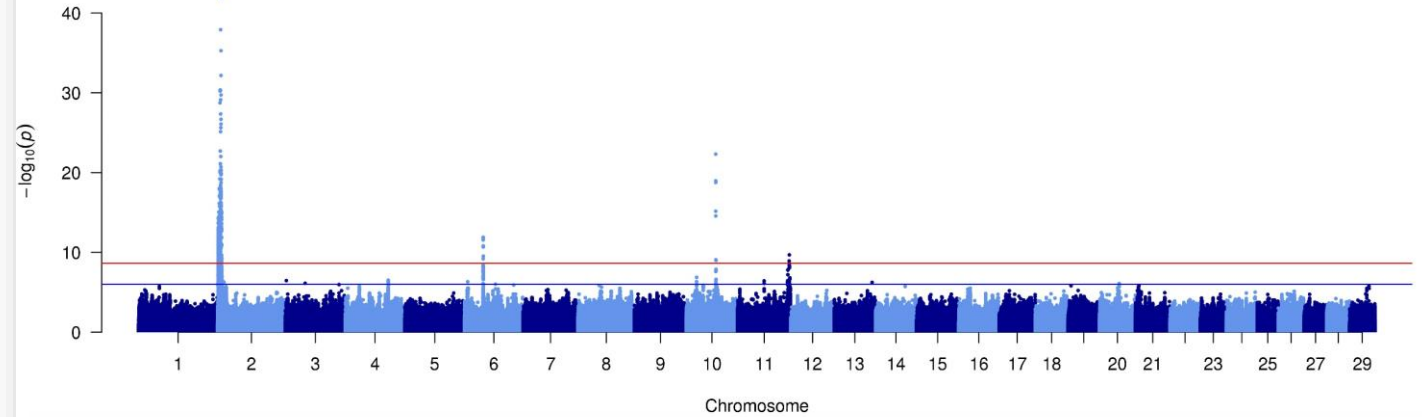
# T4.1 – « Beef » MA – results

Ex: carcass MA11

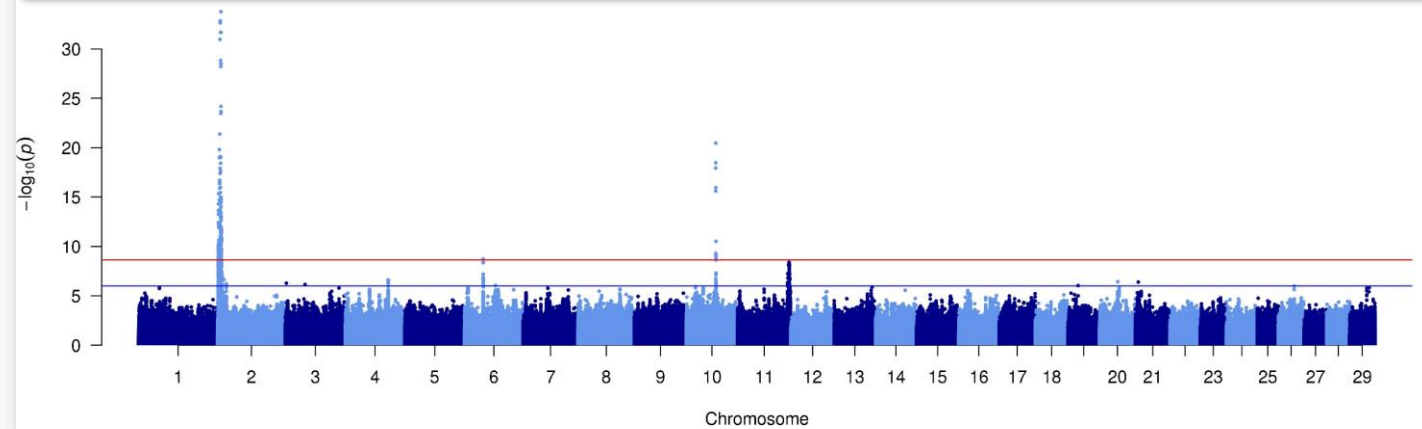
**Fixed effects vs z-score method:**

- ➔ QTL generally found with more significant effects
- ➔ More QTL detected

**Fixed effects method**



**z-score method**



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# T4.1 – « Beef » MA – results

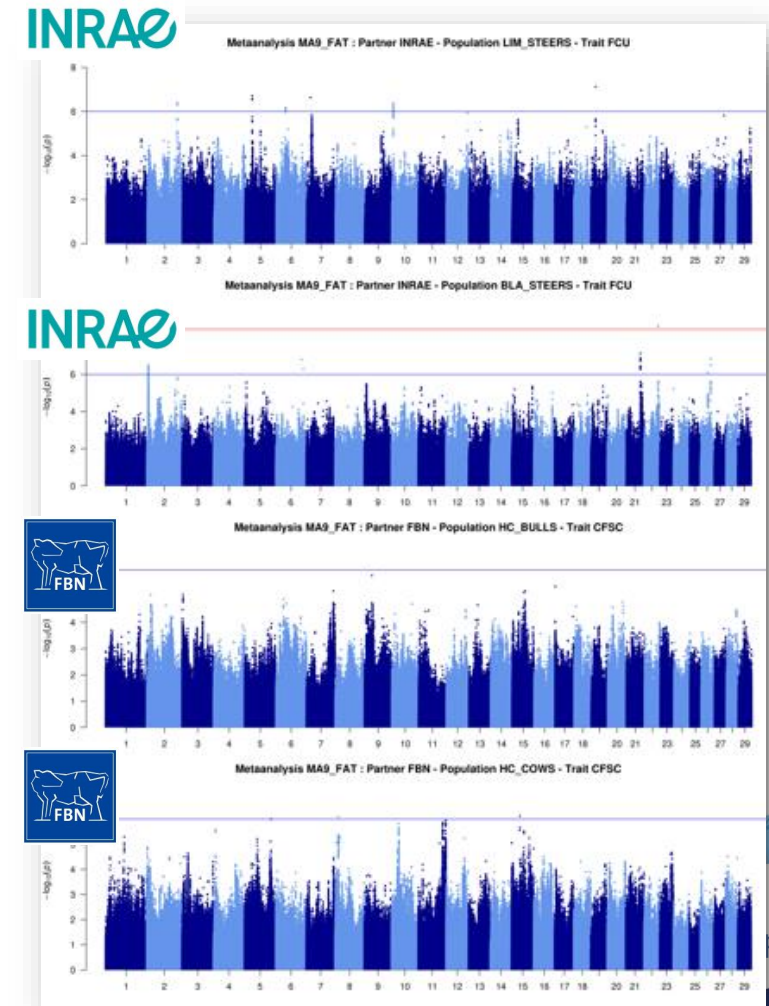
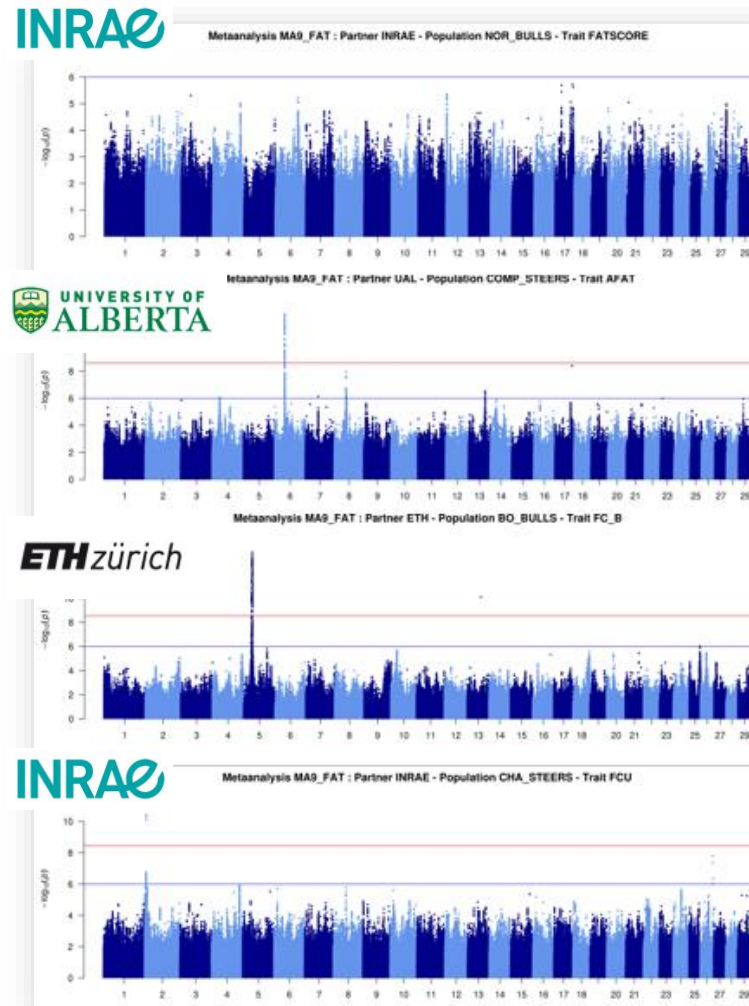
MA vs within-breed GWAS

- Confirm QTL detected in within-breed GWAS with generally more significant effects
- Detect QTL not found in within-breed GWAS

Ex: carcass MA9

**Within-breed GWAS**

=> 3 QTL on chrom. 2, 5, and 6



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# T4.1 – « Beef » MA – results

## MA vs within-breed GWAS

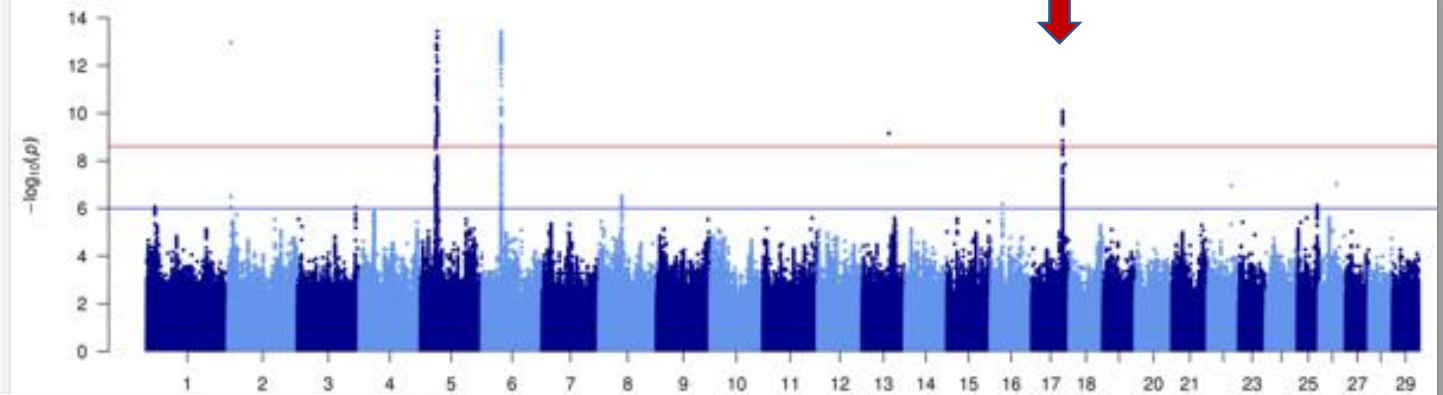
- Confirm QTL detected in within-breed GWAS with generally more significant effects
- Detect QTL not found in within-breed GWAS

Ex: Carcass MA9

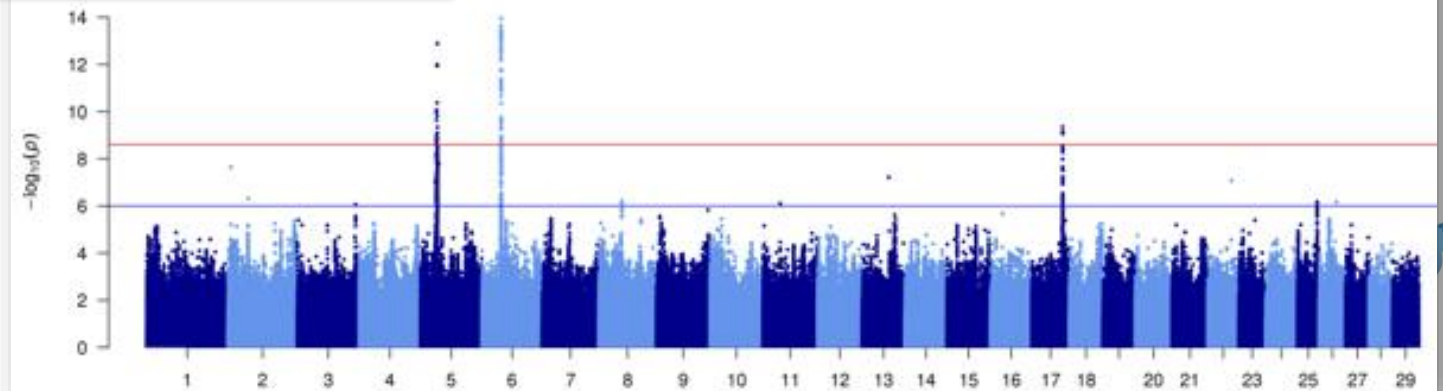
## Meta-analyses

=> 3 QTL on chrom. 2, 5, and 6  
+ 1 new QTL on chrom. 17

Fixed Effects method



z-score method



# T4.1 – « Beef » MA – results

MA vs within-breed GWAS

**Functional annotation of TOP1 variants** (variants with the most significant effects)

Functional annotation	Within-breed GWAS (%)	Fixed effects MA (%)
downstream_gene_variant	1.9	<b>13.9</b>
frameshift_variant	1.9	2.8
intergenic_region	37.7	27.8
intron_variant	39.6	36.1
missense_variant	1.9	2.8
stop_gained	11.3	11.1
synonymous_variant	1.9	0.0
upstream_gene_variant	3.8	<b>5.6</b>
<b>% TOP1 variants in genes</b>	<b>62.3</b>	<b>72.2</b>

**Do the MA help to target causal variants?**

=> Various situations depending on the QTL



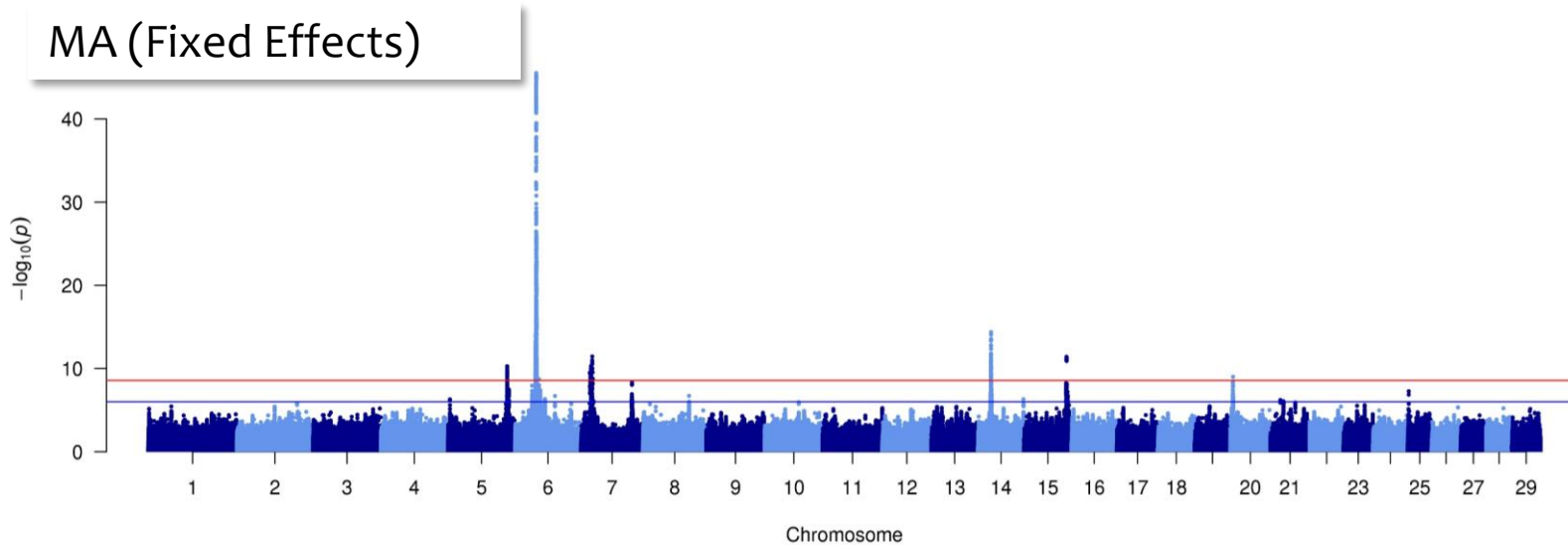
# T4.1 – « Beef » MA – results

MA vs within-breed GWAS

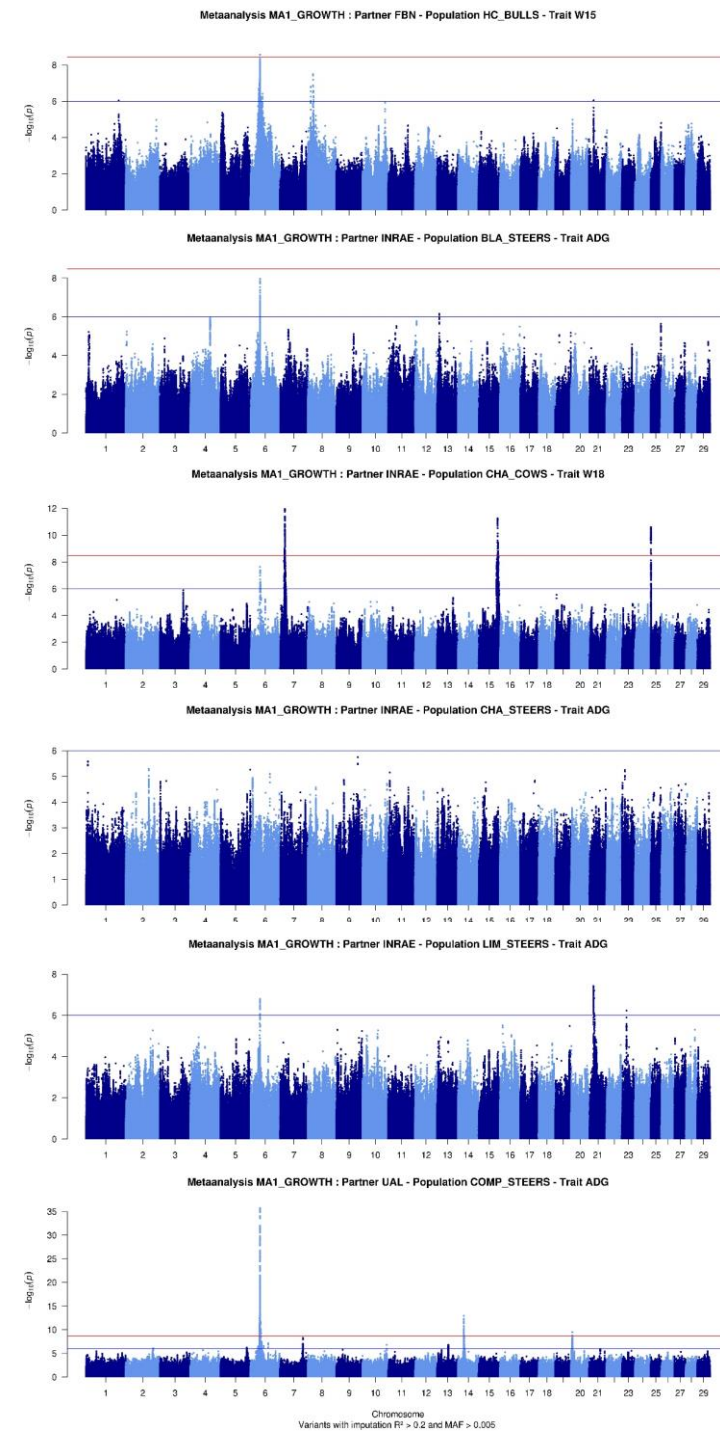
- In some cases, MA appear to better target causal variants

Ex: Growth MA1

MA (Fixed Effects)



QTL found in 10 / 16 MA



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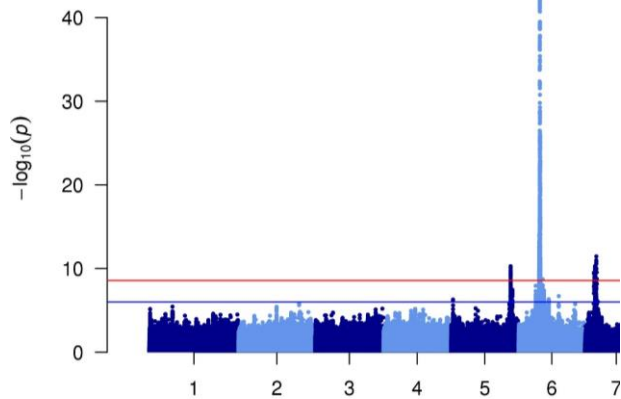
# T4.1 – « Beef » MA – results

MA vs within-breed GWAS

- In some cases, MA appear to better target causal variants

Ex: Growth MA1

MA (Fixed Effects)



QTL found in 10 / 16 MA

In all cases, the TOP1 variant is in (or near) *LCORL* ( $\neq$  within-breed GWAS)

MA	Trait type	$-\log_{10}(p)$ max	Annotation	GENE
MA1	Growth	45.5	intergenic_region	<i>LCORL-SLIT2</i>
MA2	Growth	22.0	intron_variant	<i>LCORL</i>
MA5	Morphology	37.1	intron_variant	<i>LCORL</i>
MA6	Morphology	21.1	intron_variant	<i>LCORL</i>
MA7	Morphology	18.0	intron_variant	<i>LCORL</i>
MA8	Carcass	31.4	intergenic_region	<i>LCORL-SLIT2</i>
MA11	Carcass	11.9	frameshift_variant	<i>LCORL</i>
MA12	Carcass	14.7	intron_variant	<i>LCORL</i>
MA13	Carcass	29.2	intron_variant	<i>LCORL</i>
MA16	Carcass	18.1	missense_variant	<i>LCORL</i>

***LCORL*** => transcription factor

Associated to different traits (growth, carcass, stature, ingestion...)  
in various populations (e.g. Doyle et al., 2020)

If 1 causal mutation shared between breeds => less extent LD  
between breeds (MA) can help to better target the causal mutation



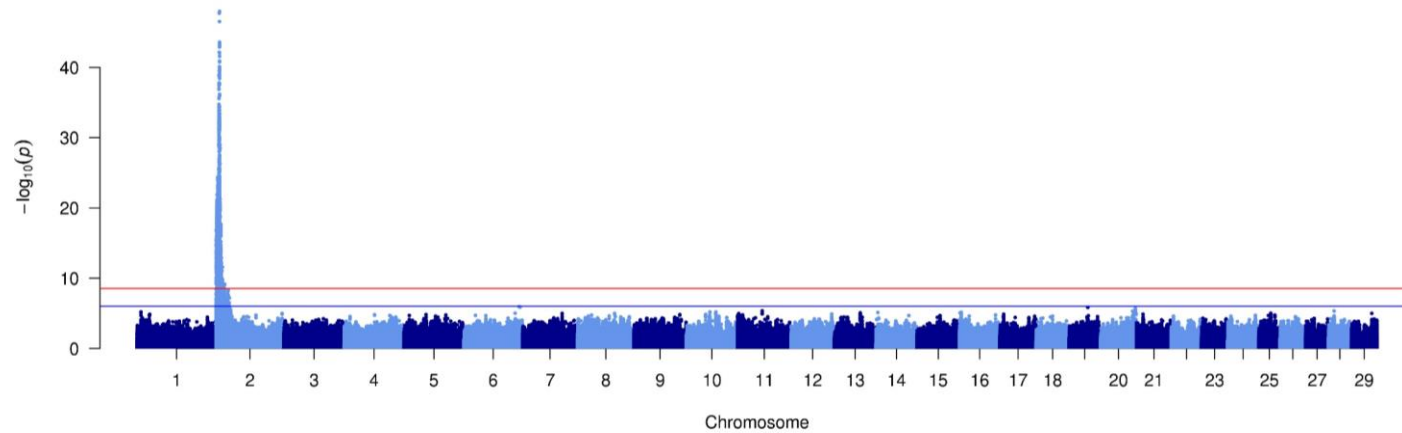
# T4.1 – « Beef » MA – results

MA vs within-breed GWAS

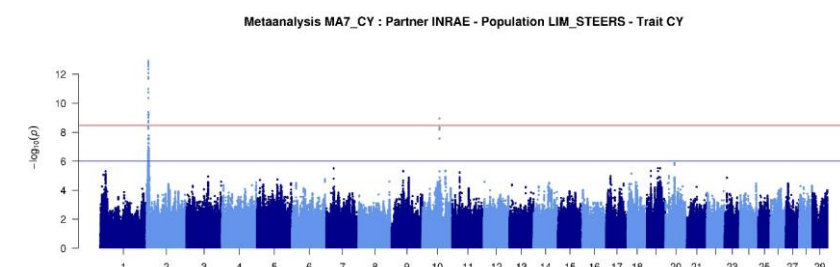
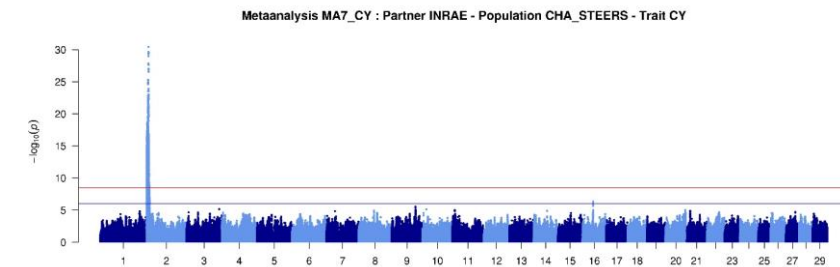
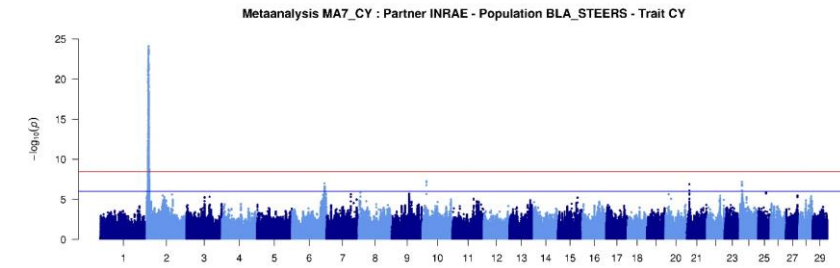
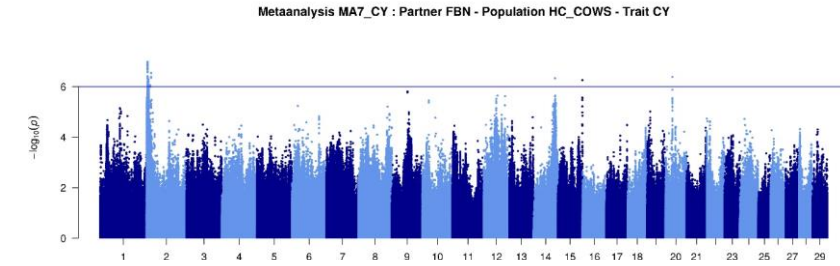
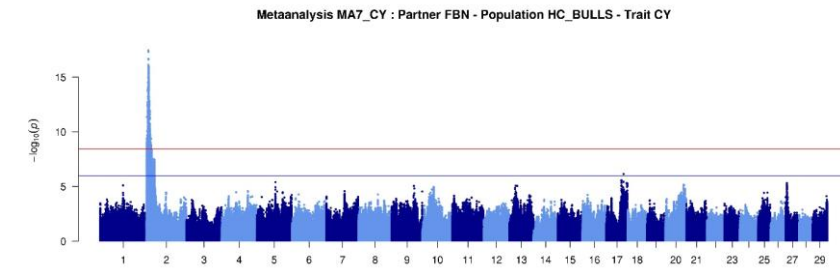
- In other cases, MA appear to « dilute » the signal

Ex: Carcass MA10 (carcass yield)

MA (Fixed Effects)



Larger confidence interval in MA than in some within-breed GWAS



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# T4.1 – « Beef » MA – results

MA vs within-breed GWAS

- In other cases, MA appear to « dilute » the signal

Chromosome 2

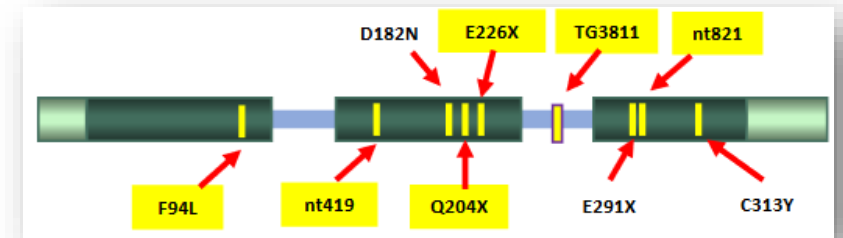
**MSTN** gene (myostatin)

Muscular hypertrophy



McPherron and Lee, 1997

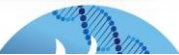
9 known mutations in *MSTN*



The most frequent mutation differs depending on the breed (Renand et al., 3R 2020)

Allelic frequencies	n	+	F94L	Q204X	nt419	E226X	TG3811	nt821
Salers	1 099	99,6	0,1					0,2
Charolais	42 780	84,2	6,5	9,2				
Aubrac	775	8,1	87,0	0,2			2,0	2,6
Parthenais	1 350	0,4	0,2	0,3	4,3	7,6	0,3	86,9
Blonde d'Aquitaine	16 343	0,2	0,5	0,2	0,6		98,5	
Limousine	11 677	0,1	99,1	0,2				0,5

If different causal mutations in the different breeds  
=> The GWAS signal can be diluted



# T4.1 – « Beef » MA – discussion / conclusions

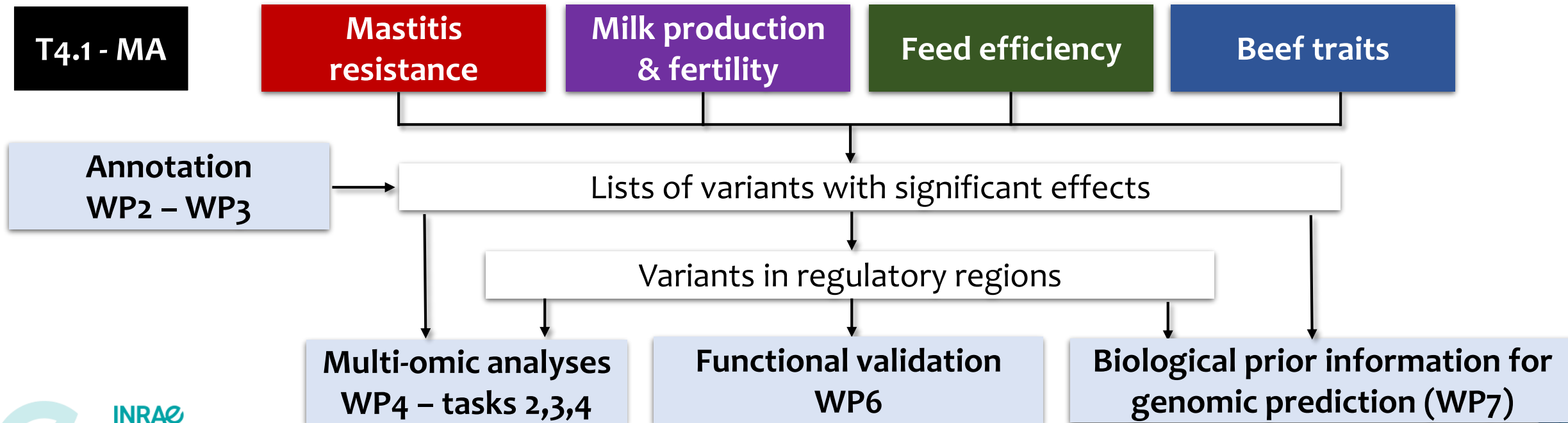
**Meta-analyses** conducted from within-breed GWAS results (without elementary data)

=> QTL with more significant effects (+ new QTL)

=> Appear to better target causal variants in some cases (shared mutation)

New investigations have to be conducted to identify genes and causal variants

Links with other BovReg tasks/WP



# KO BovReg meeting, Sept. 2019



*Merci!*



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