Genes and variants involved in resistance to paratuberculosis in Holstein and Normande cattle

V. Sorin[1], A. Boulling[1], A. Delafosse[2], M. Boussaha[1], C. Hozé[1,3], R. Guatteo[4], C. Fourichon[4], S. Fritz[1,3], D. Boichard[1] & M.P. Sanchez[1]

[1] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas
[2] GDS Orne, 61000 Alençon
[3] Eliance, 75595 Paris
[4] INRAE, Oniris, BIOEPAR, 44300 Nantes
France

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Paratuberculosis in cattle

Paratuberculosis or Johne's disease

Transmissible endemic disease in ruminants due to *Mycobacterium avium subsp. paratuberculosis* (**MAP**)

Very frequent: ½ herds infected in Europe

Gut lesions & rapid weight losses

Strong economic impact
& negative effects on animal welfare

No efficient treatment & vaccine available

Long latency period: young animals infected, first positive tests after 2-6 years

Poor sensitivity/specificity of detection tests (ELISA blood / PCR feces)

Difficult to control MAP

Objectives

Identify genes and variants affecting resistance to MAP

GWAS & meta-GWAS at the whole genome sequence level in Holstein and Normande cows

Phenotypes & Genotypes

Cows with MAP statuses from 2 datasets

❶ Blood serological / fecal PCR tests
PARADIGM project, Sanchez et al. GSE 52:14, 2020

❷ Blood serological tests routinely recorded since 2015, Sanchez et al. GSE 54:67, 2022

Rigorous selection of non infected cows

Not in latency period (> 3 years-old)

Exposed to MAP, from herds with at least one contemporary infected cow

Holstein		**Normande**
65,836	Cows with MAP statuses	31,083
4677	Incl. cows with 50k chip genotypes	**4845**
3277	Controls (non infected)	3578
1263	Subclinical cases	1195
137	Clinical cases	72

Methods & tools for GWAS and meta-GWAS

Within-breed imputations

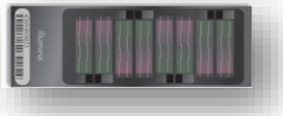
Fimpute / Minimac

50k

Step 1

776 Holstein & **546 Normande** animals with HD SNPs

HD



Step 2

3414 animals of various breeds with WGS incl. 1414 Holstein and 160 Normande (RUN9 1000 BG + INRAE)

WGS

17 million variants after QC for 4677 Holstein 4845 Normande

GWAS & meta-GWAS

GCTA / METAL

Within-breed GWAS: each variant tested together with a polygenic effect estimated from a 50k GRM

Meta-analyses by combining within-breed GWAS results (fixed effects method)

Significant effects if $-\log_{10}(\text{p-value}) > 7.3$

Results: heritability of resistance to paratuberculosis

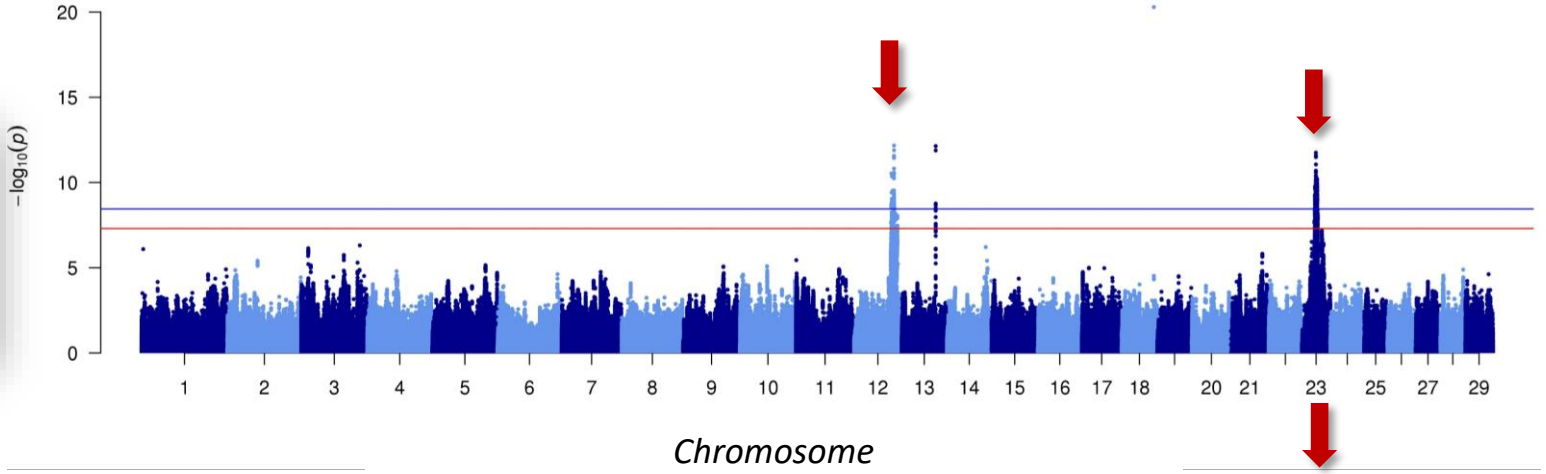
Estimated based on the 50k genomic relationship matrix in the population

$h^2 = 0.32$

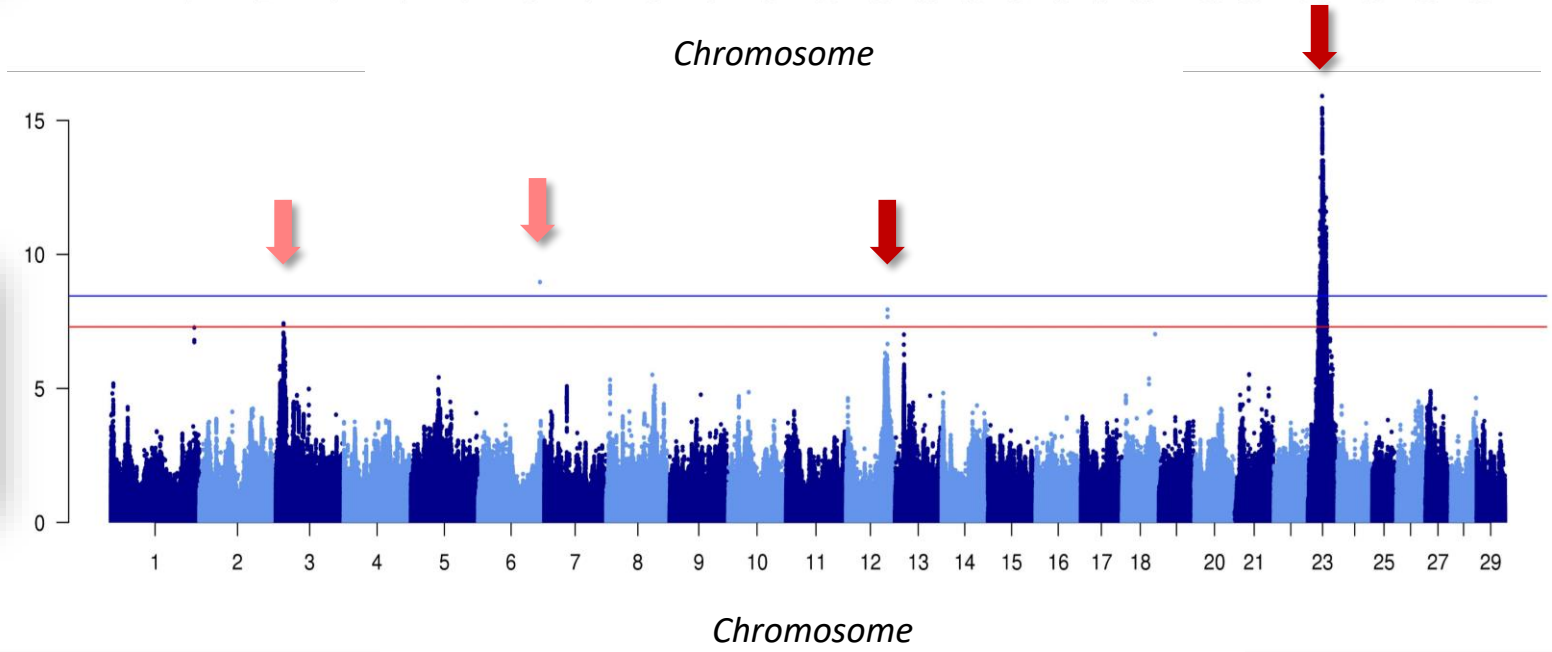
$h^2 = 0.25$

Moderate / medium values suggesting a genetic determinism of the host resistance to MAP

Results: within-breed GWAS



4 regions
Chr. **12**, 13, 18, **23**

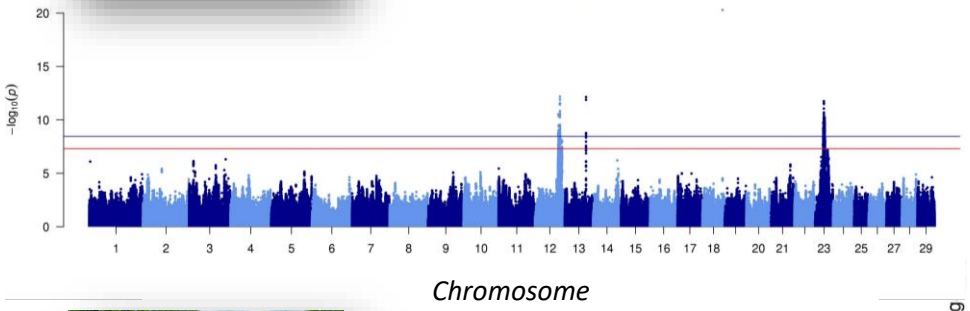


4 regions
Chr. 3, 6, **12**, **23**

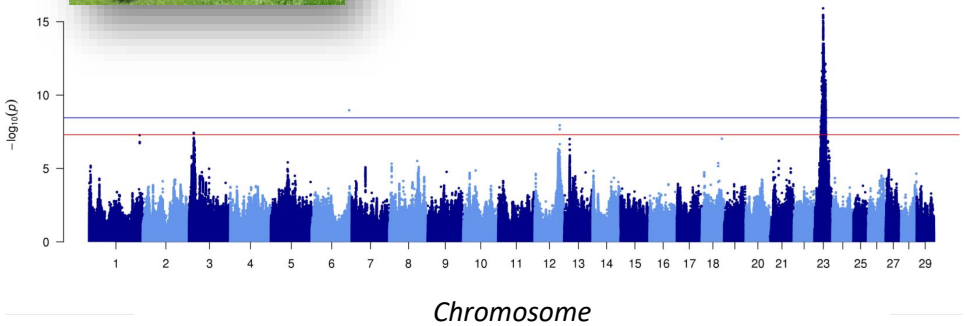
4 regions in each breed, two are located on chromosomes **12** and **23** in both breeds

Results: meta-GWAS

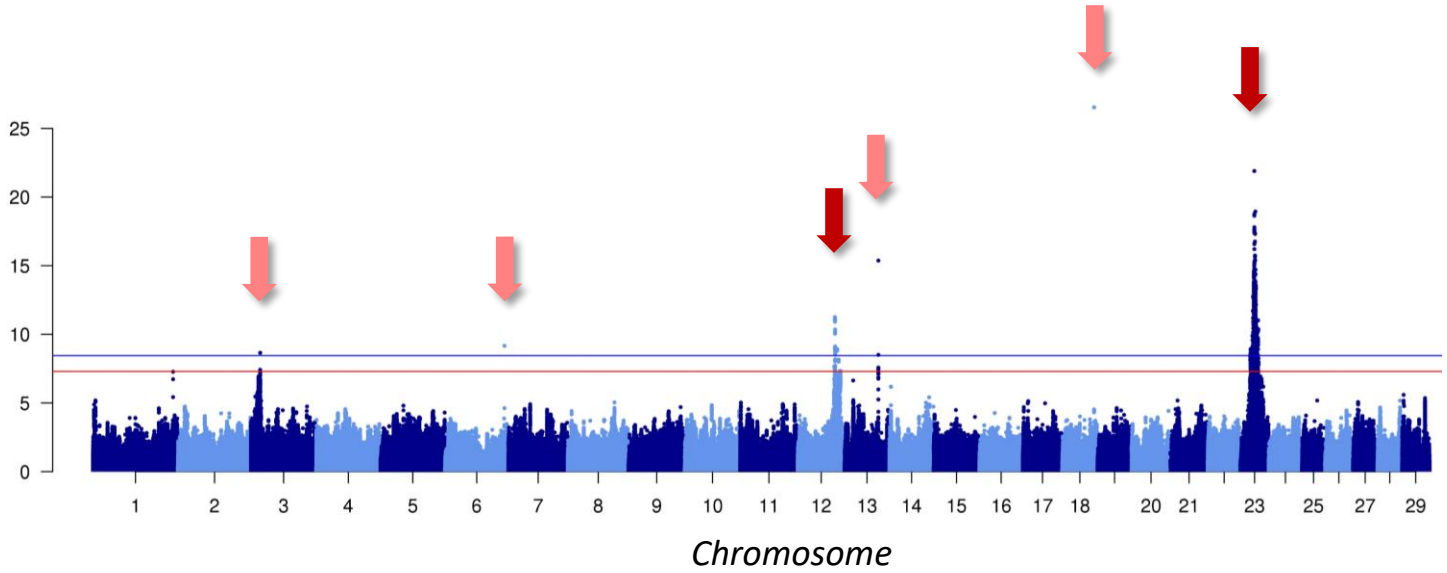
4 regions
Chr. **12**, 13, 18, **23**



4 regions
Chr. 3, 6, **12**, **23**



6 regions with meta-GWAS
Chr. 3, 6, **12**, 13, 18, **23**



Common + **specific** regions
More regions than each within-breed GWAS with more significant effects (except on chr. 13)

Definition of the QTL regions

In each region, with an **iterative procedure** based on **LD** between variants, applied in 5-Mb intervals centered around the lead variant

- Single or multi QTL within a genomic region
- Confidence interval for each QTL identified

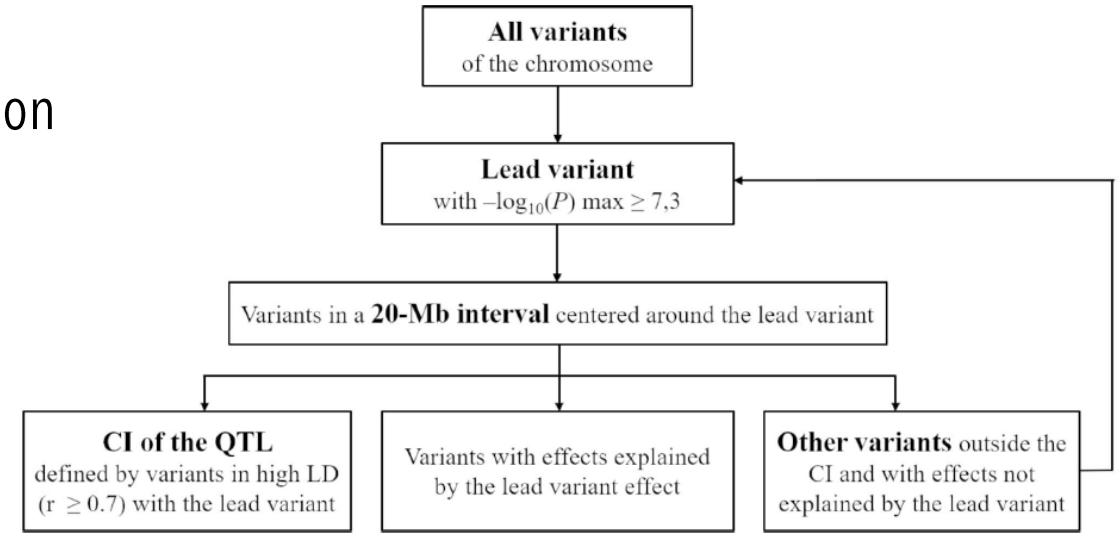


Fig. 1 Iterative procedure for defining QTL and their confidence intervals

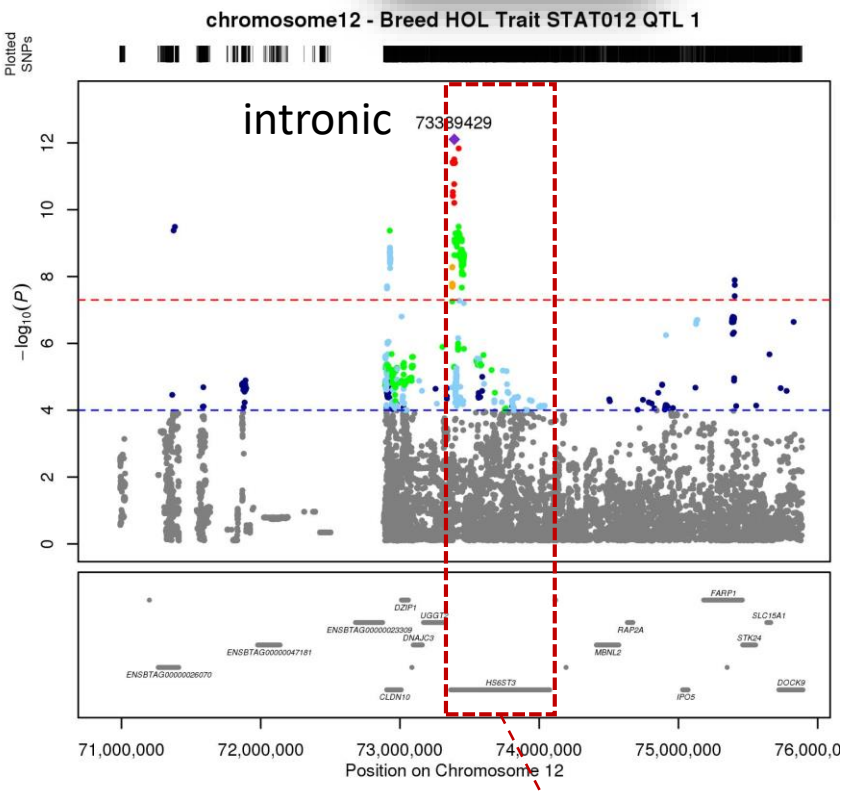
Sanchez et al. (2023) BMC Genomics, 24:338
https://doi.org/10.1186/s12864-023-09438-7

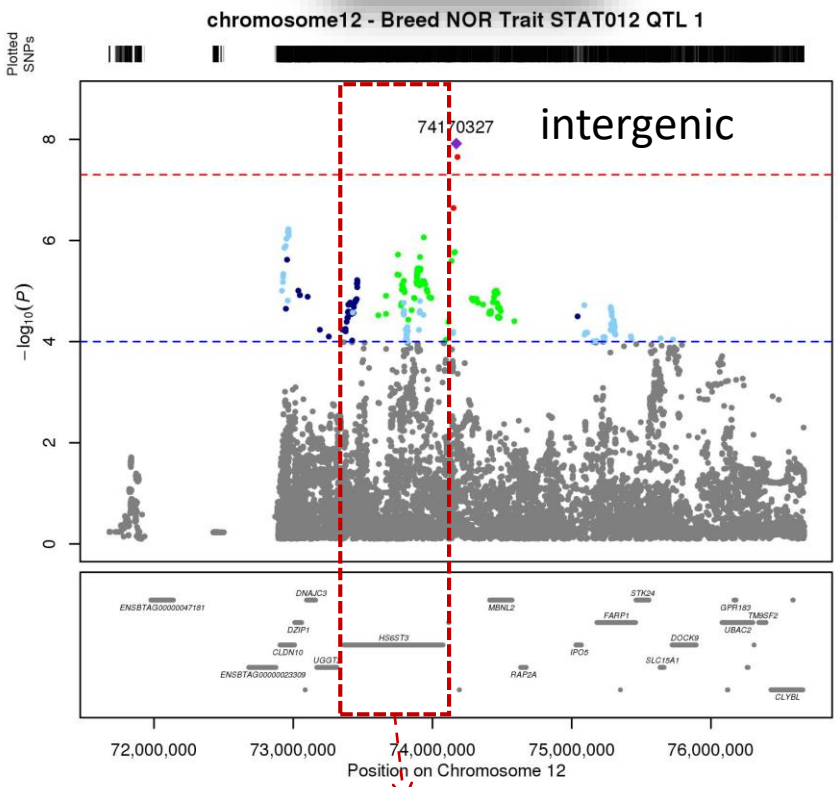
QTL identified in within-breed and meta-GWAS results

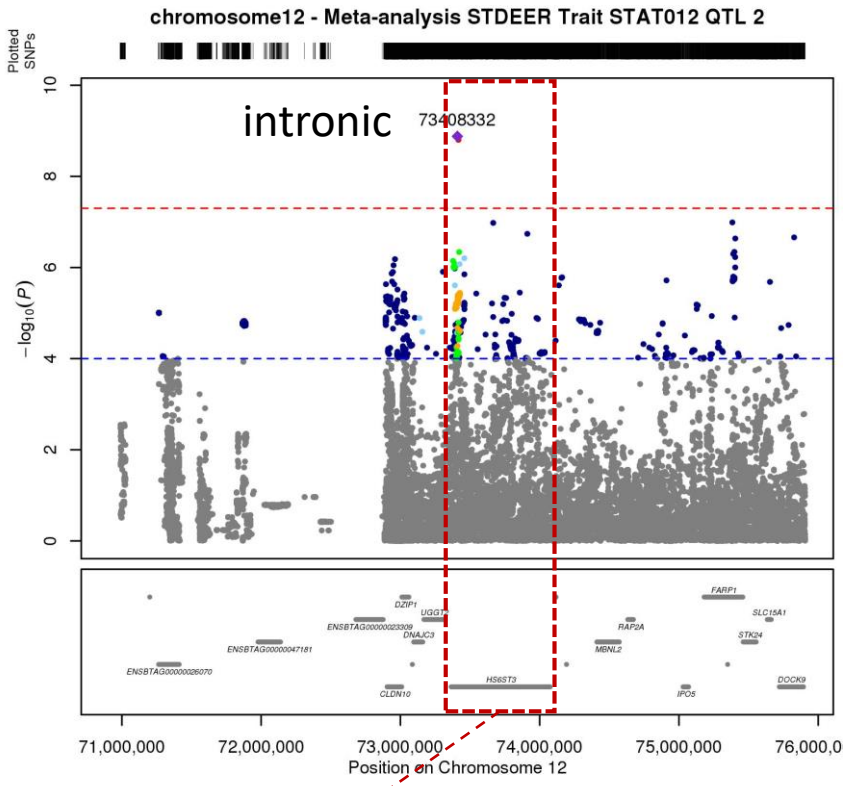
	Chr. 3	Chr. 6	Chr. 12	Chr. 13	Chr. 18	Chr. 23	Total
Normande	1	1	1	0	0	5	**8**
Holstein	0	0	4	1	1	3	**9**
Meta-GWAS	1	1	4	2	1	15	**24**

Results: QTL on chr. 12

Meta-GWAS



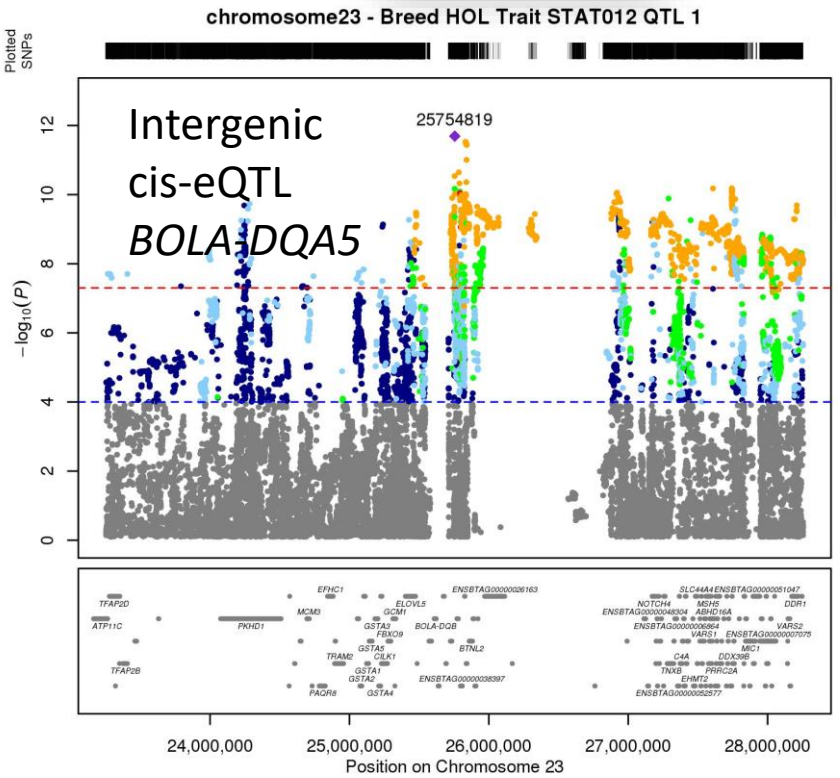


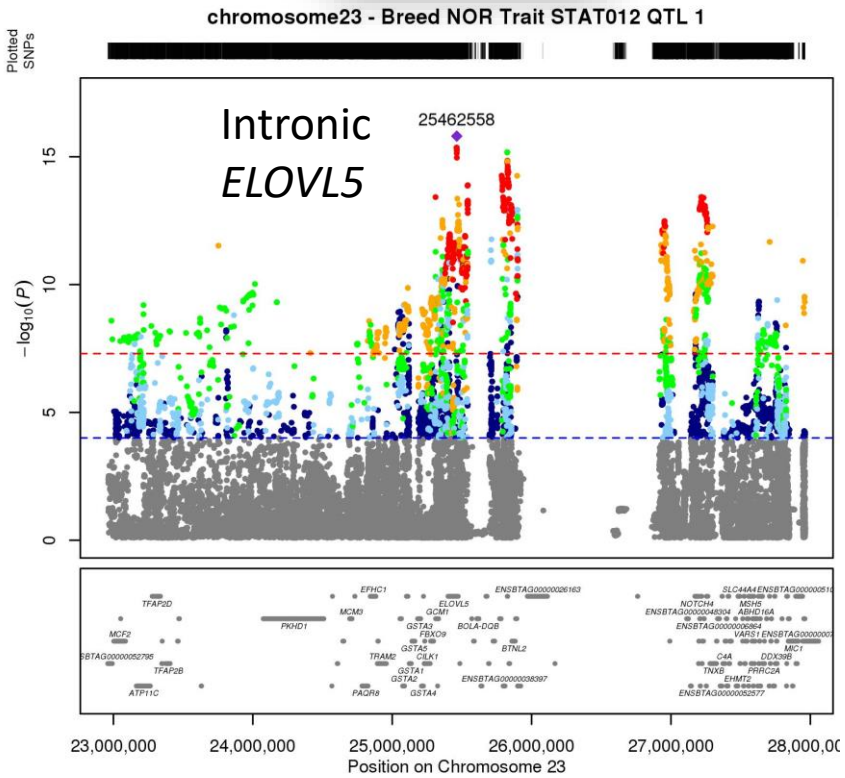


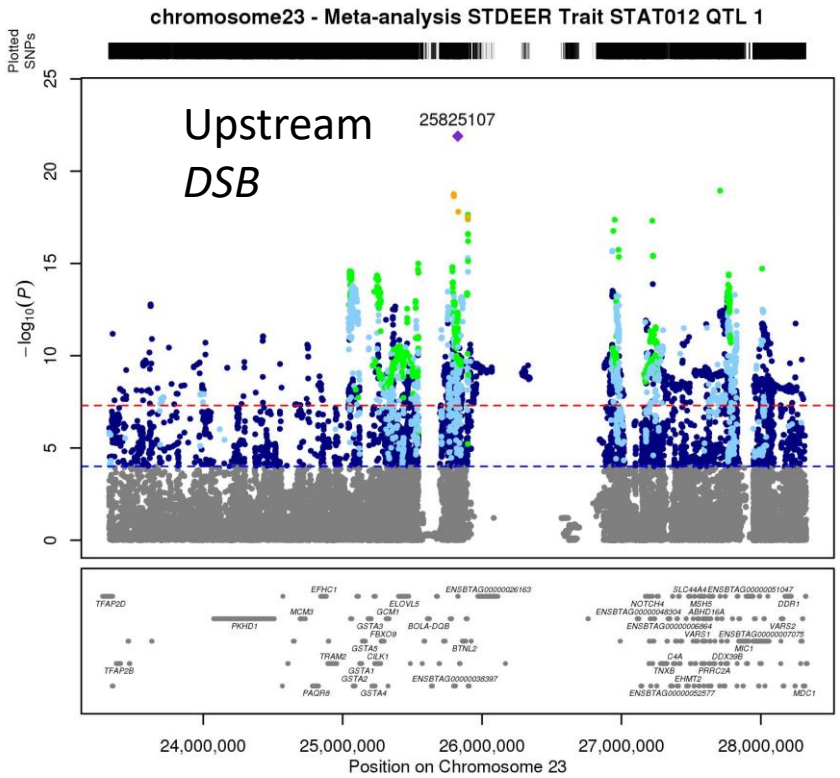
HS6ST3 is a 700 kb gene
expressed mainly in brain tissues
& involved in various processes, incl. inflammation

Results: QTL on chr. 23

Meta-GWAS







MHC = complex region with many genes and a high level of polymorphism
Meta-GWAS clearly reduced LD in the region but different variants / genes could be responsible for the effects observed

Results: 3 candidate variants / genes on chr. 23

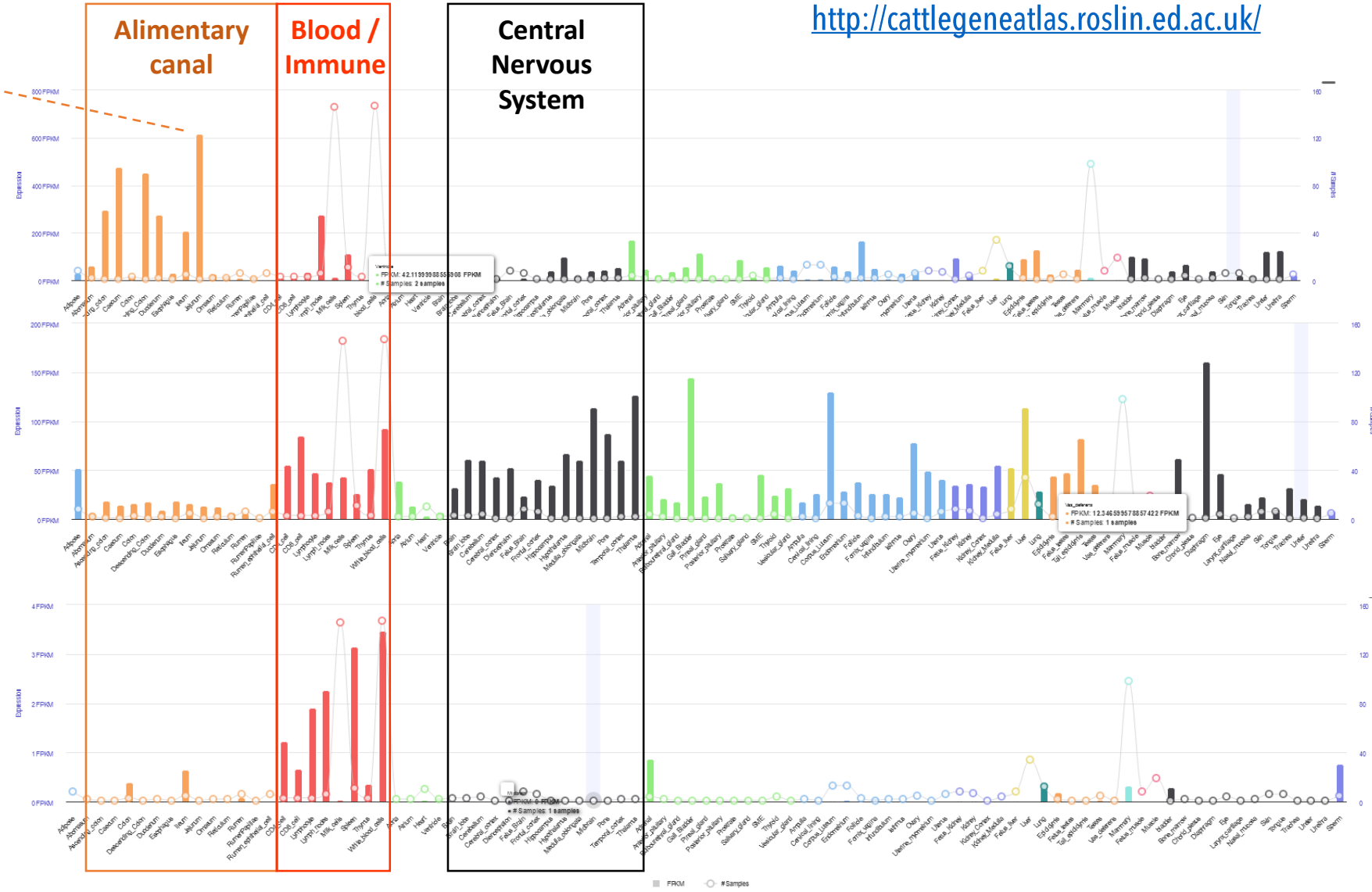
Jejunum where lesions caused by MAP are observed

Intergenic cis-eQTL
BOLA-DQA5

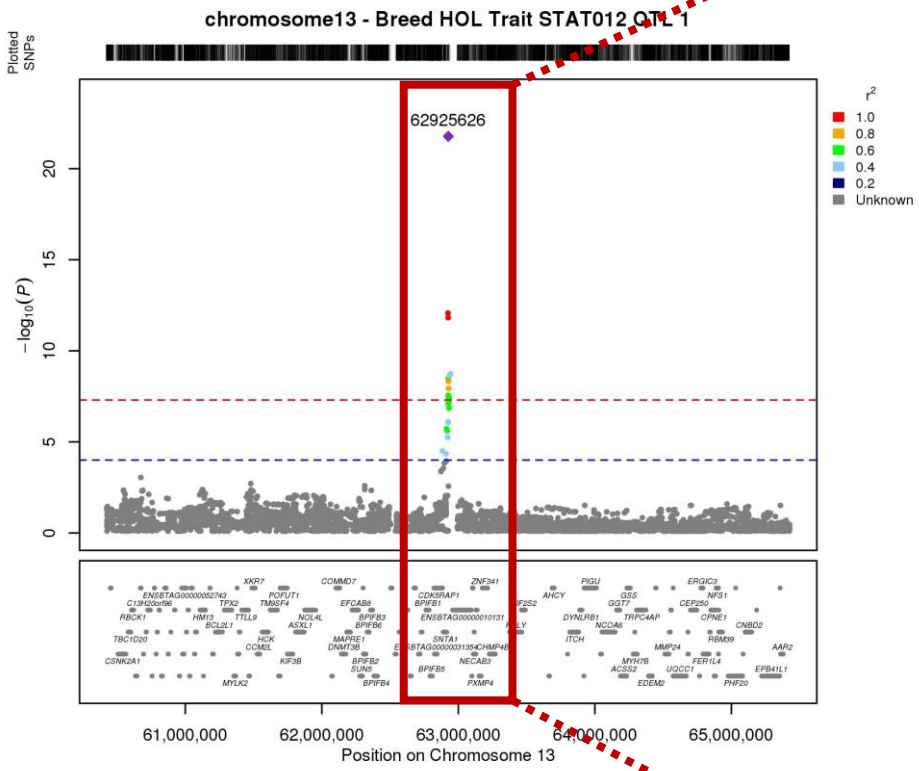
Intronic *ELOVL5*

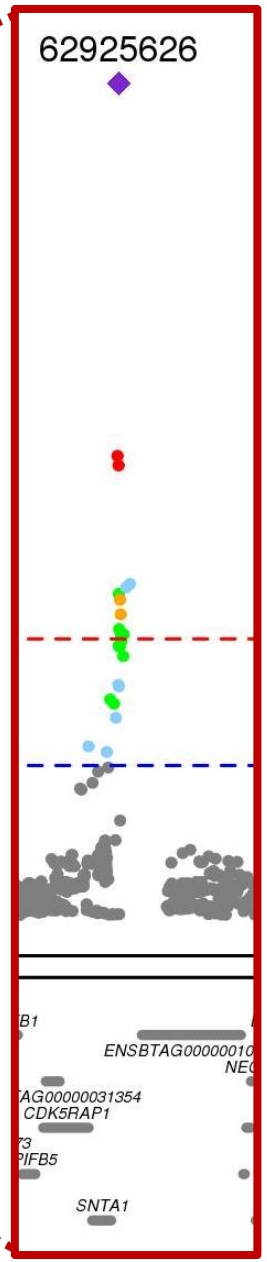
Meta-GWAS

Upstream *DSB*



Results: QTL on chr. 13 (specific to Holstein?)

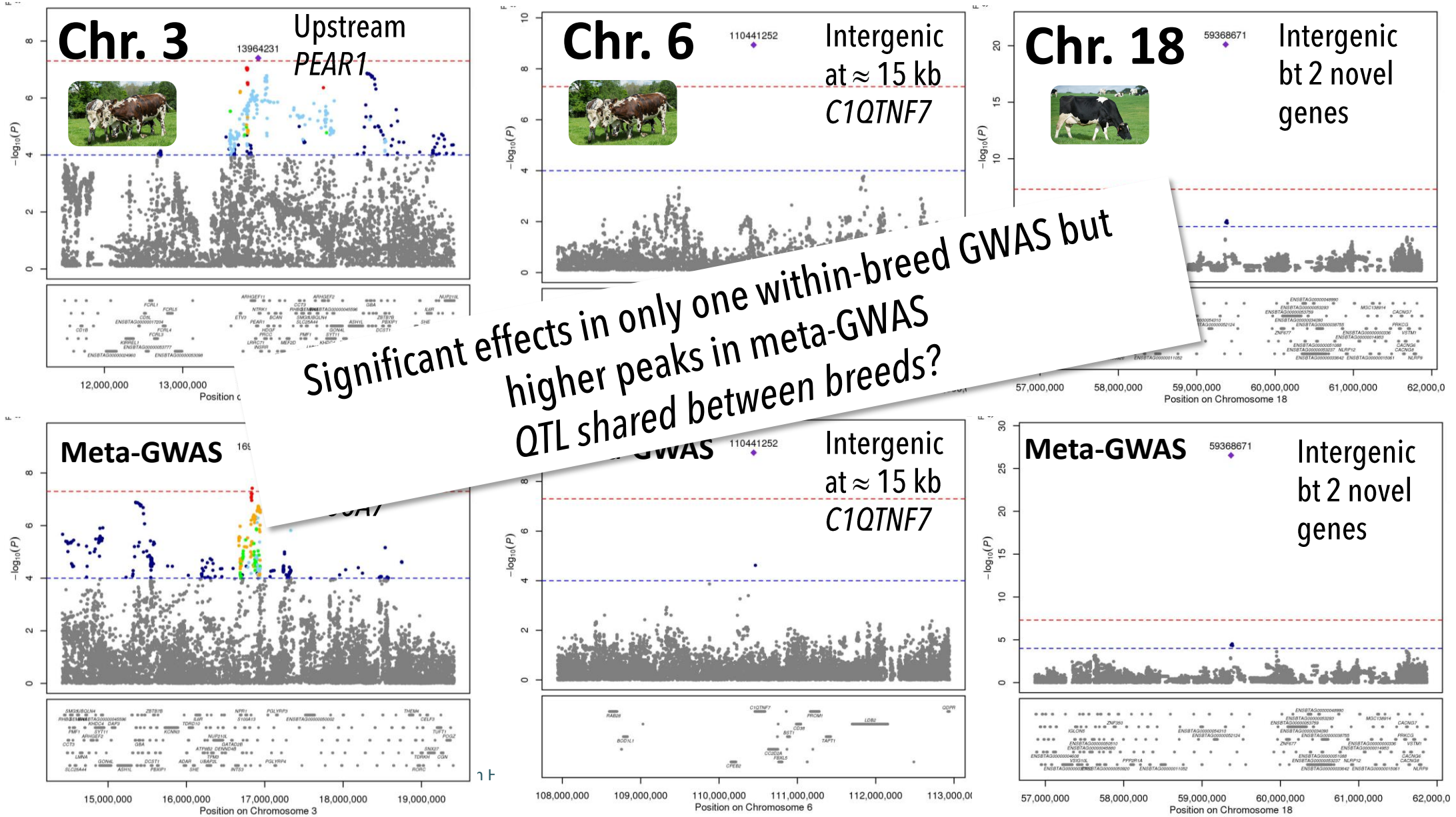




Weaker signal with meta-GWAS

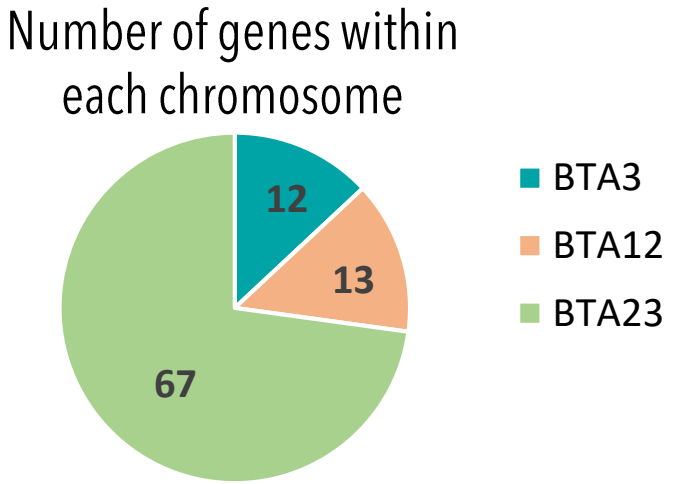
The lead variant is an intergenic variant between *SNTA1* & *CBFA2T2*

Results: QTL on chr. 3 and 6 in Normande / chr. 18 in Holstein



Results: Enrichment analyses

In meta-GWAS, identification of **799 variants** located in **92 genes** on chr. 3, 12, and 23



Enrichment analyses based on different public human databases:

	PheWeb 2019	GWAS Catalog 2019	UK Biobank GWAS v1
Bowel diseases	Celiac disease	Ulcerative colitis, Inflammatory bowel disease	Malabsorption/coeliac disease, Other diseases of the digestive system
Auto-immune diseases	Type 1 diabetes, Psoriasis	Atopic march	Adrenocortical insufficiency/Addison's disease, Psoriasis *(Medication dovonex)*

=> Enrichment for diseases related to paratuberculosis

Conclusions

This study identifies QTL for resistance to paratuberculosis from GWAS and meta-GWAS at the WGS level on 4845 Normande and 4677 Holstein cows

Overall, meta-GWAS confirm all QTL detected in each within-breed GWAS with more significant effects & suggest that most QTL could be shared between breeds

Identification of variants and genes being good functional candidates for resistance to paratuberculosis with an enrichment of ontologies related to bowel diseases

Selection of the best functional candidate variants for validation

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