



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

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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



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> 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

GWAS & meta-GWAS

GCTA / METAL

Step 1

50k



776 Holstein & 546 Normande
animals with HD SNPs

HD



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

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 both populations



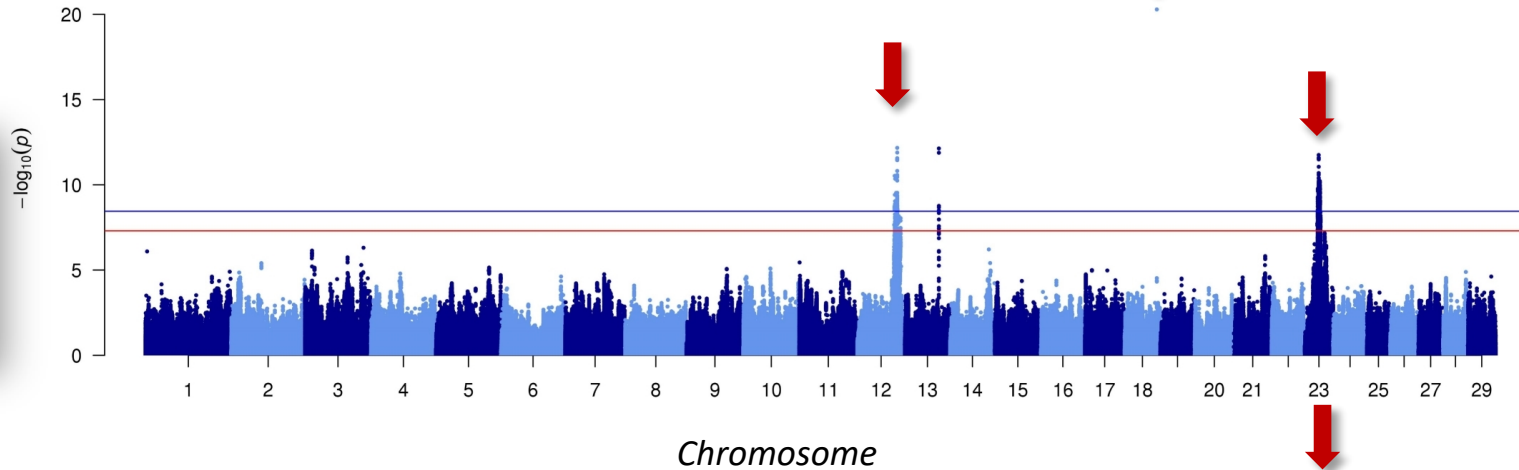
$$h^2 = 0.32$$



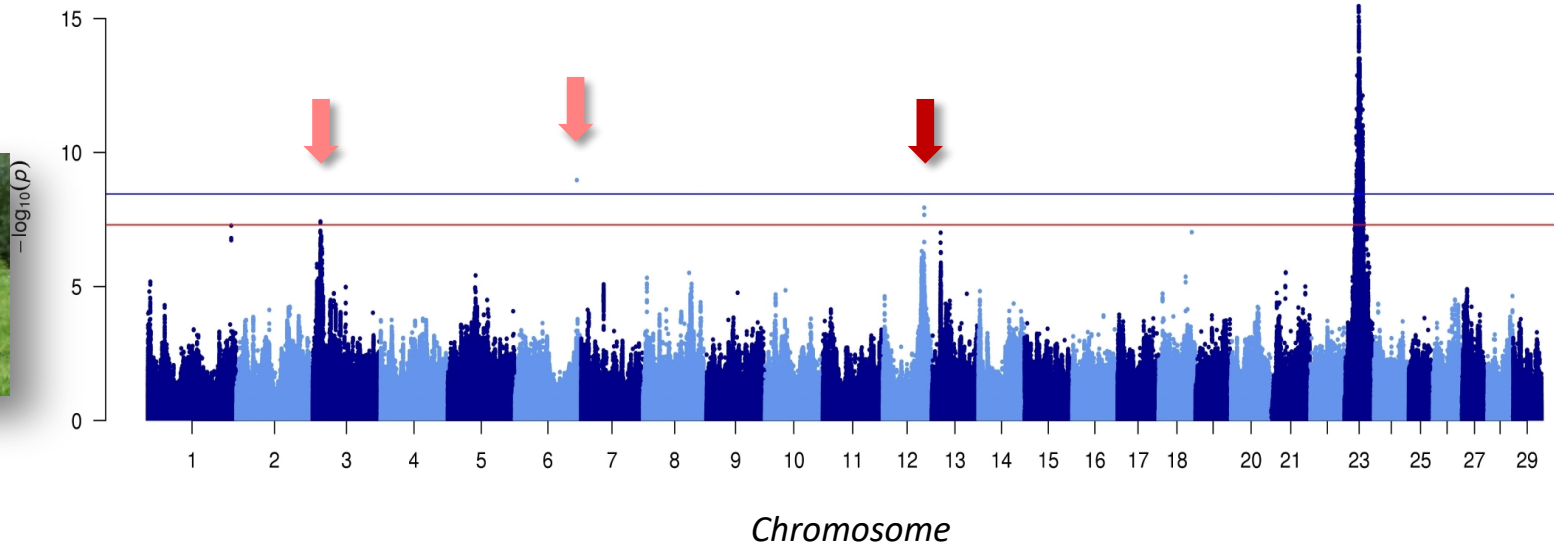
$$h^2 = 0.25$$

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

➤ Results: within-breed GWAS



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



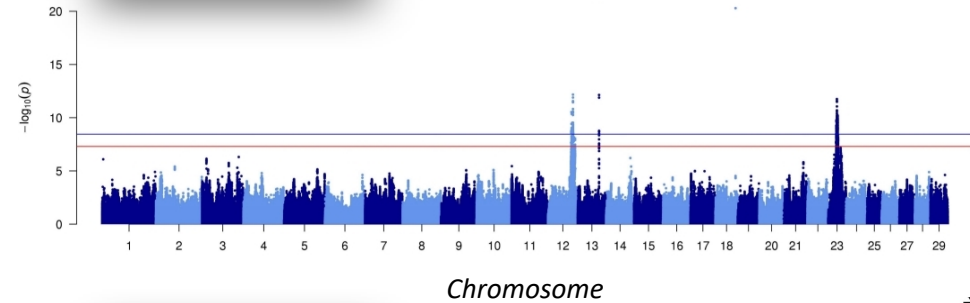
4 regions
Chr. 3, 6, **12**, **23**

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

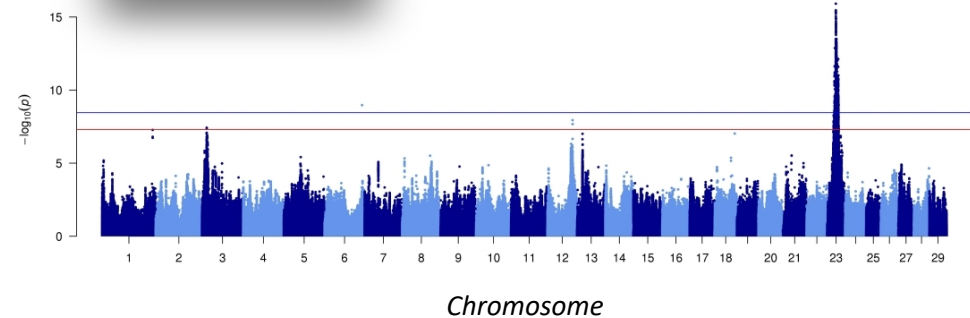
➤ Results: meta-GWAS



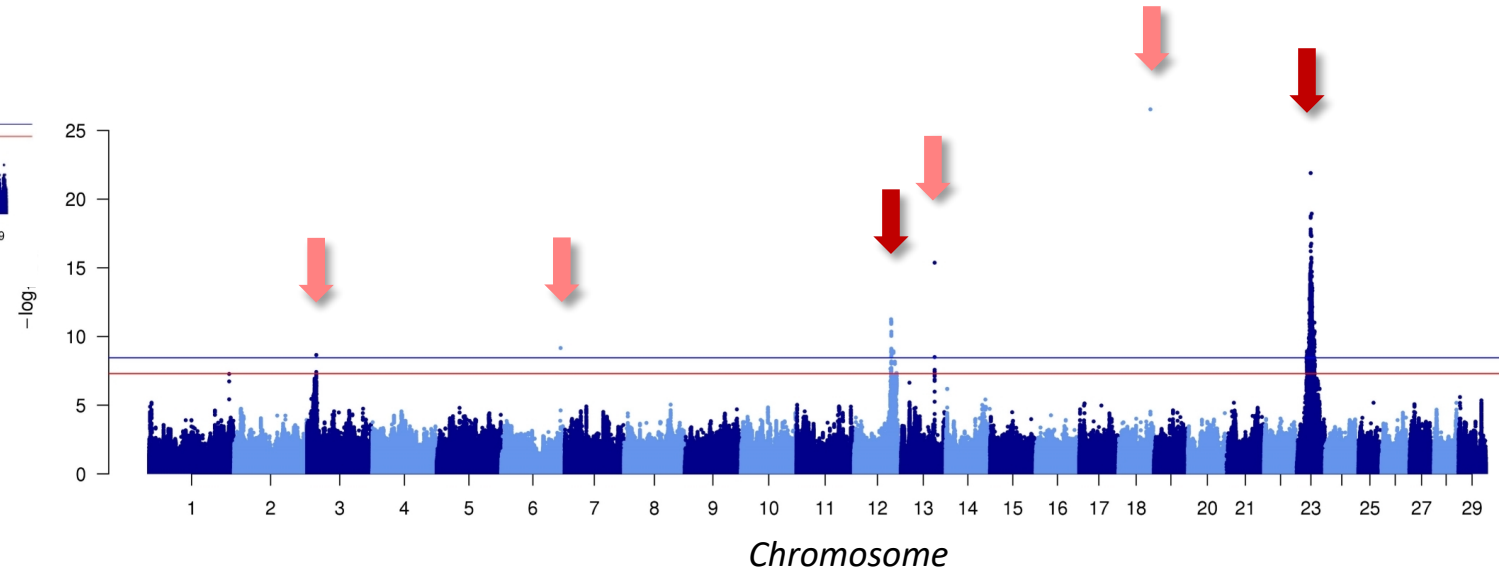
4 regions
Chr. 12, 13, 18, 23



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)



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> 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

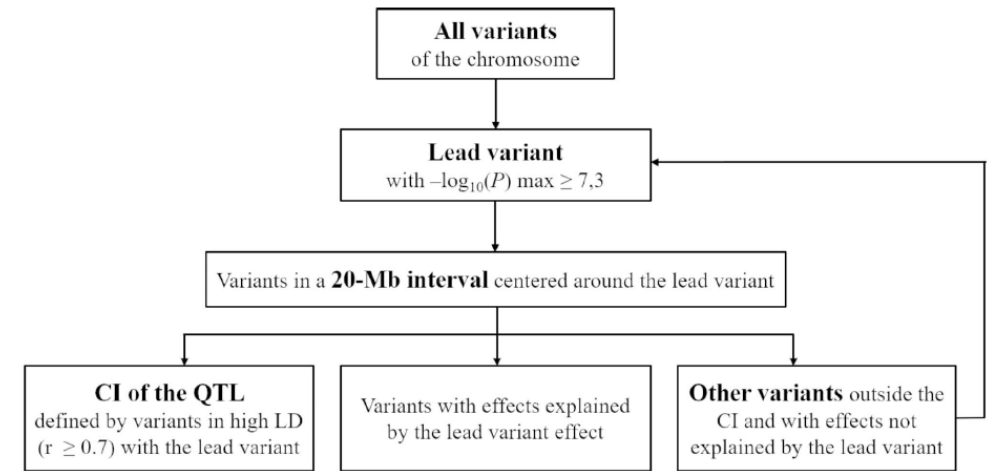


Fig. 1 Iterative procedure for defining QTL and their confidence intervals

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

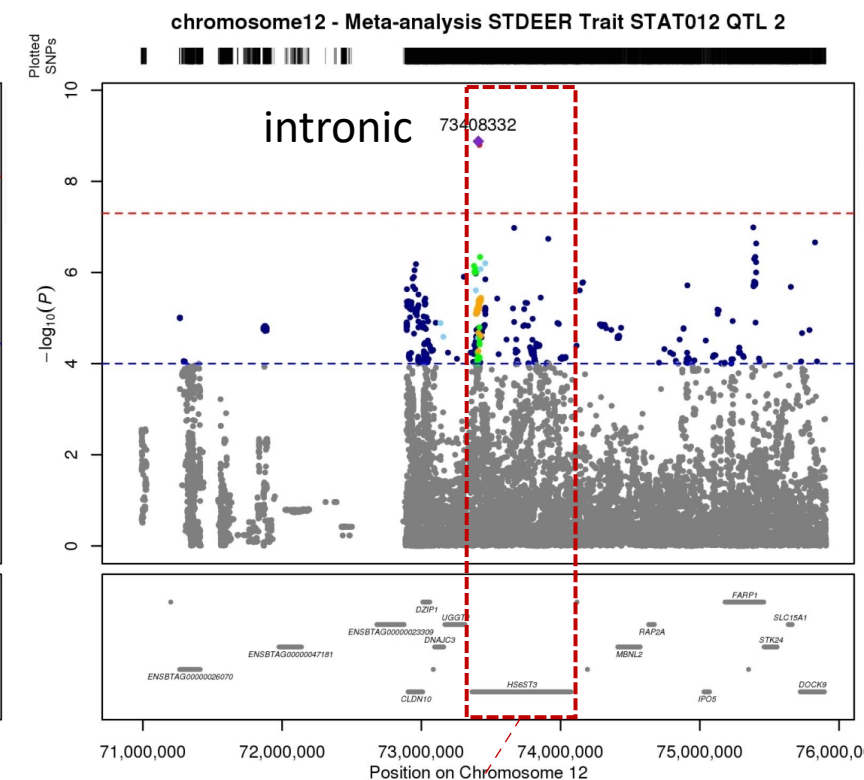
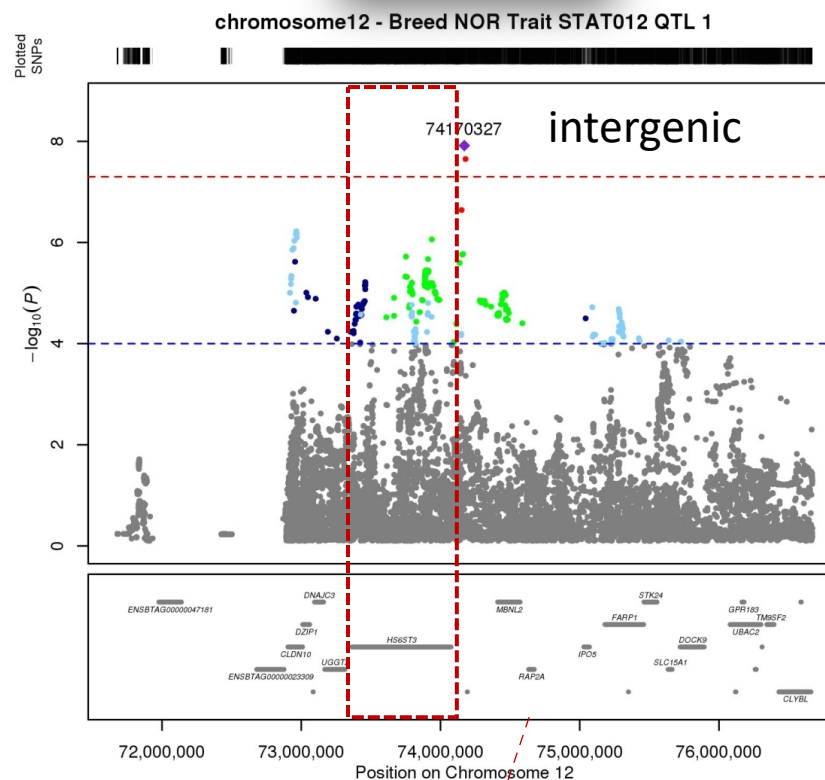
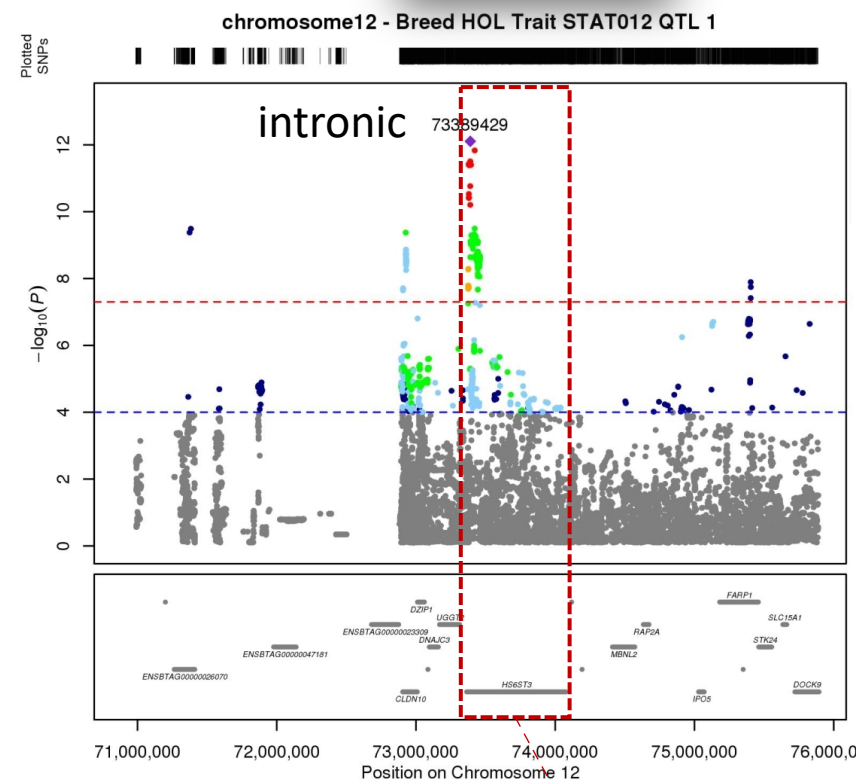
QTL identified in within-breed and meta-GWAS results

	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

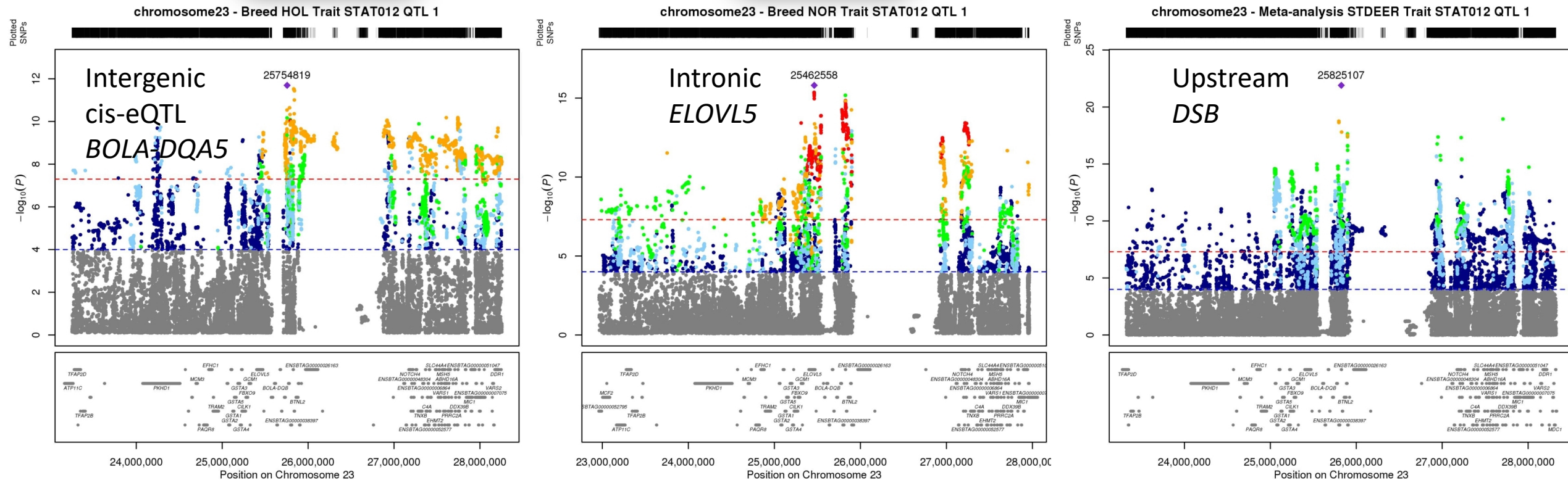


HS6ST3 is a 700 kb gene
expressed mainly in brain tissues
& involved in various processes, incl. inflammation

➤ 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

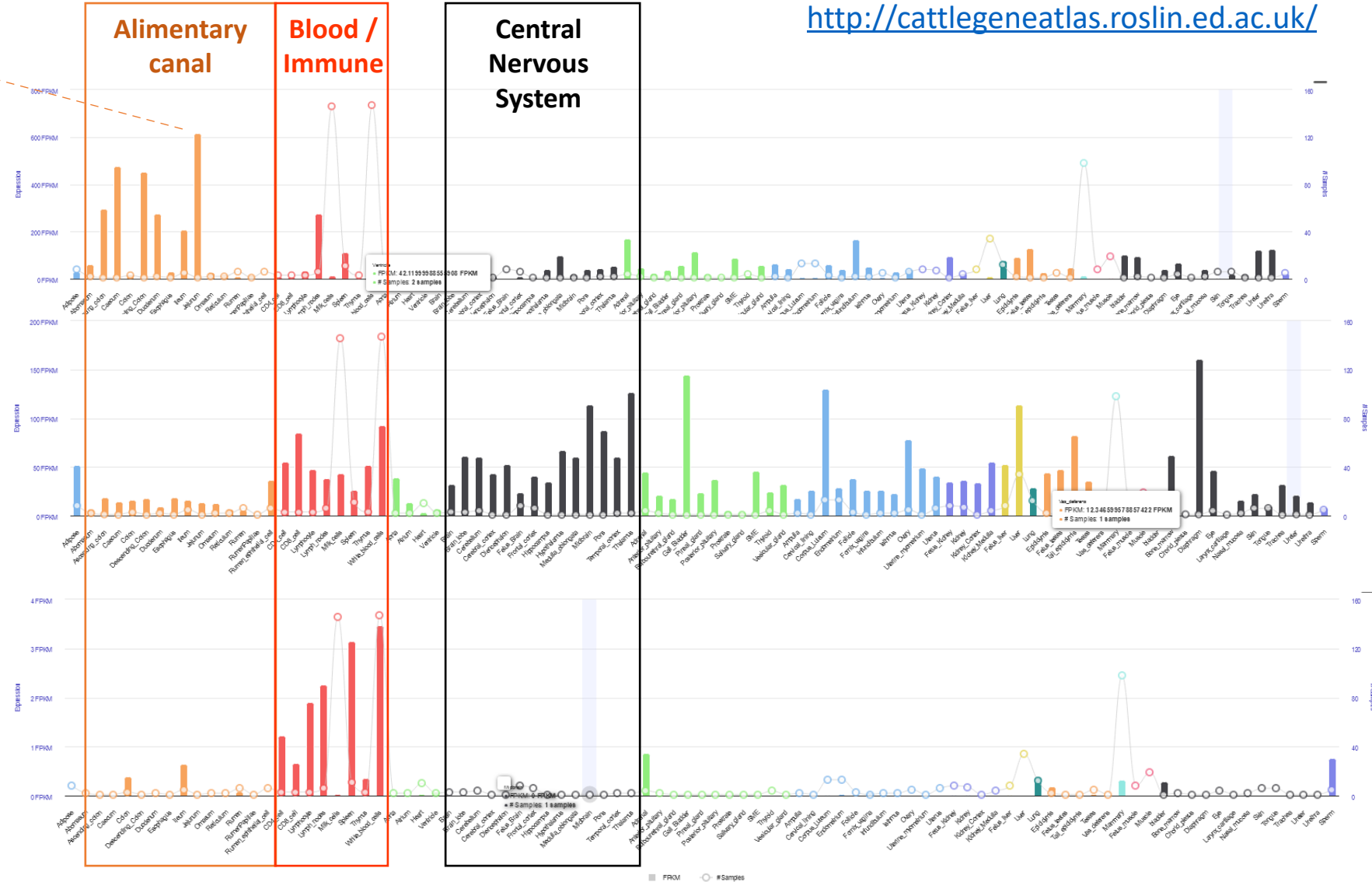


Intronic *ELOVL5*

Meta-
GWAS

Upstream *DSB*

<http://cattlegeneatlas.roslin.ed.ac.uk/>

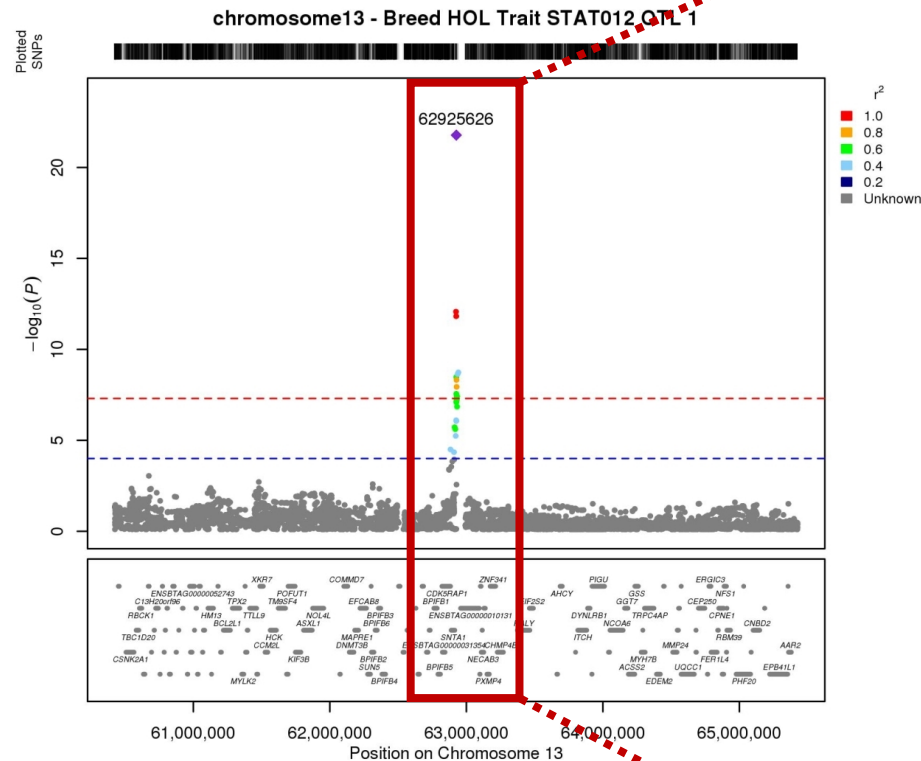


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➤ Results: QTL on chr. 13 (specific to Holstein?)



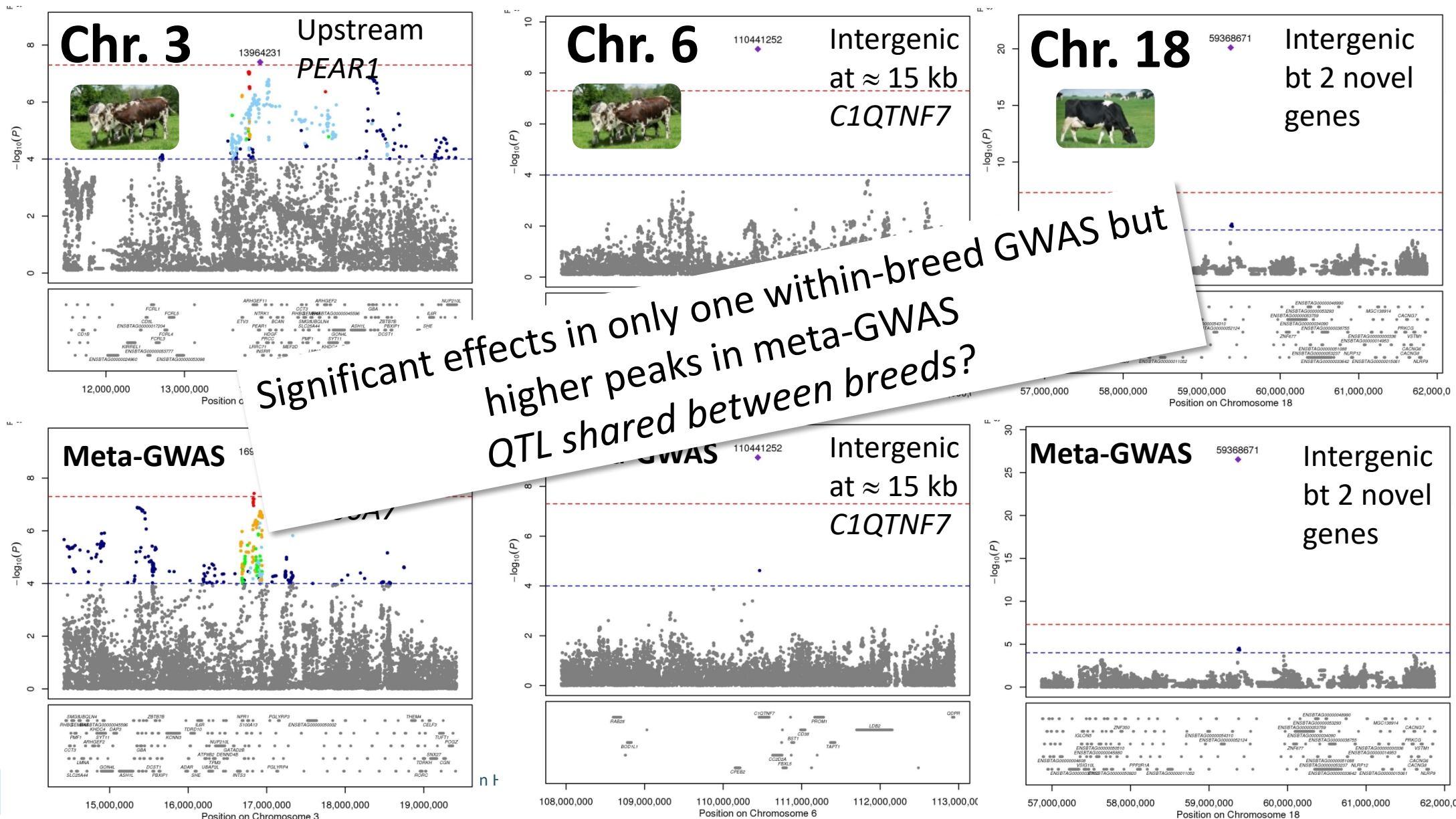
Weaker signal with
meta-GWAS

62925626

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

B1
ENSBTAG000000010
NE
AG000000031354
CDK5RAP1
3
IFB5
SNTA1

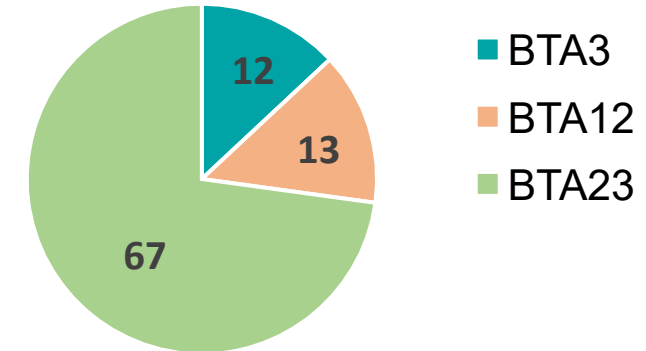
➤ Results: QTL on chr. 3 and 6 in Normande / chr. 18 in Holstein



> Results: Enrichment analyses

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

Number of genes within each chromosome



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 (<i>Medication dovonex</i>)

=> 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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