Genomic analysis of hoof and leg conformation traits in Swiss dairy cattle identified a *HYAL1*-related recessive form of polysynovitis

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Importance and genetic architecture of hoof and leg conformation traits

- **Background**
  - Poor hoof/leg conformation → higher risk of hoof disorders
- **Challenge**
  - Hoof disorders reduce animal welfare, longevity, and profitability
  - Hoof-health traits: not routinely recorded and hard to quantify
- **Opportunity**
  - Hoof and leg conformation traits are routinely scored
  - Low to moderate $h^2 = [0.05\text{-}0.37]$
  - Several GWAS regions already reported

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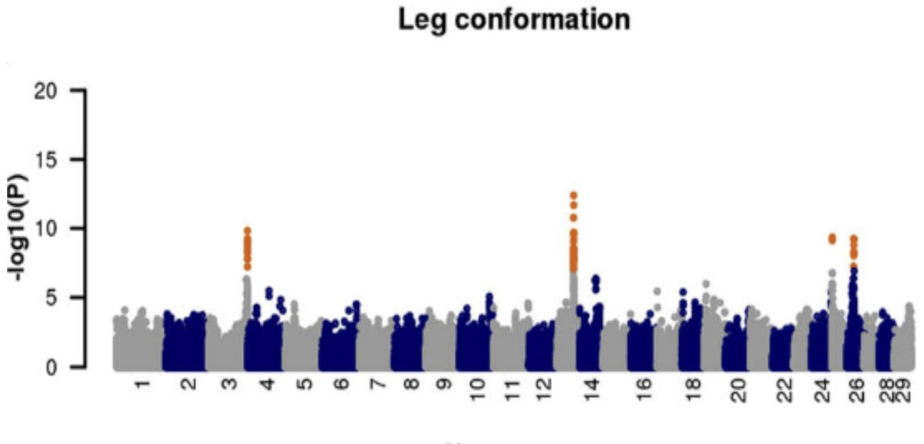

Fang and Pausch (2019)

Aims

1. Estimate the additive heritability (h^2)
2. Identify associated genomic regions using additive and non-additive model
3. Detect functional candidate genes and candidate variants
4. Characterize the phenotypic effects of the prioritized candidate variant

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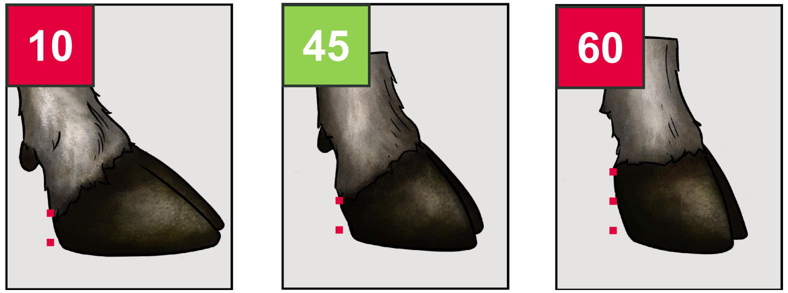
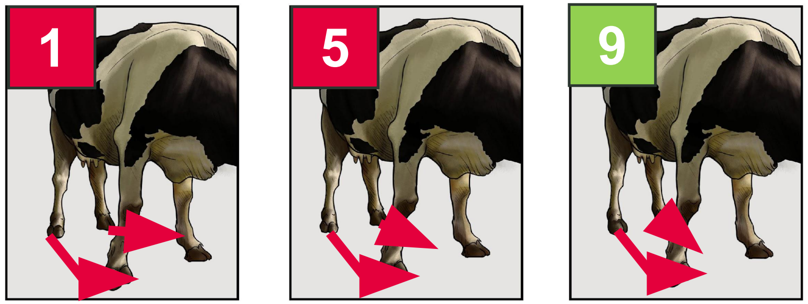
Hoof and leg conformation traits in HO

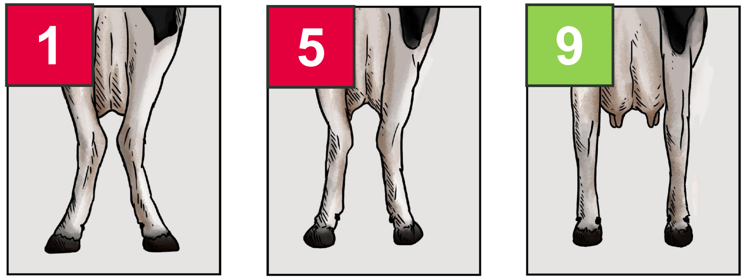
Heel depth (HDE)
millimeter: 10 to 60



Locomotion (LOC)
score: 1 to 9

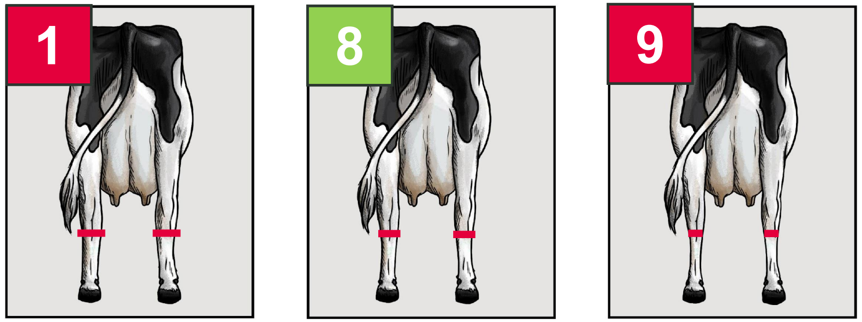


Rear leg rear view (RLR)
score: 1 to 9



Foot angle (FAN)
score: 1 to 9

Bone structure (BST)
Score: 1 to 9

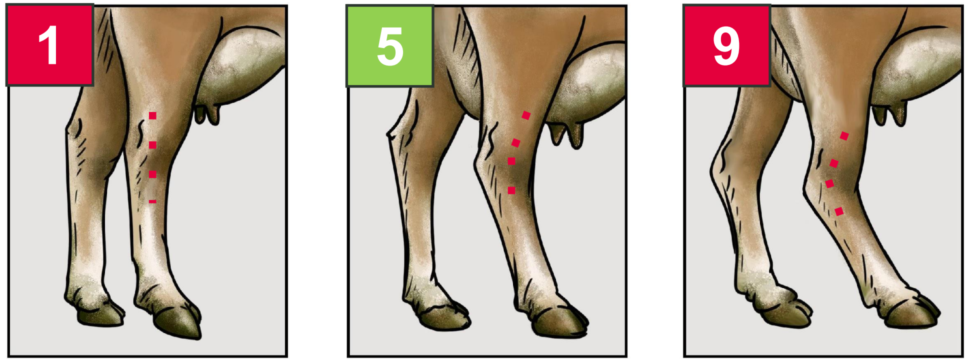


Rear leg set (RLS)
score: 1 to 9

Hoof and leg conformation traits in BS

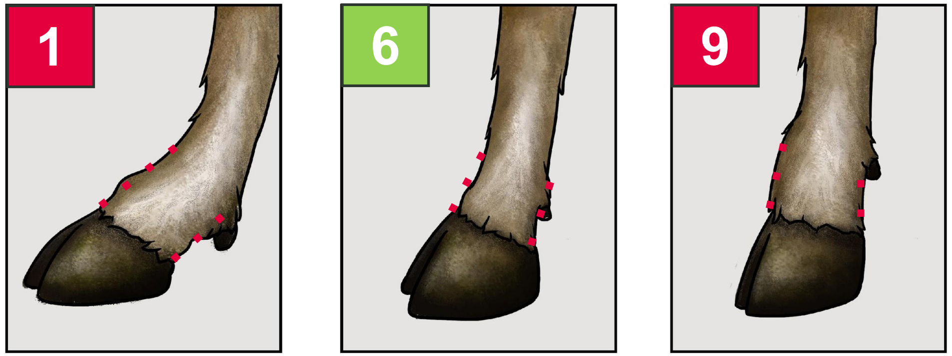
Rear leg side view (RLV)

Score: 1 to 9



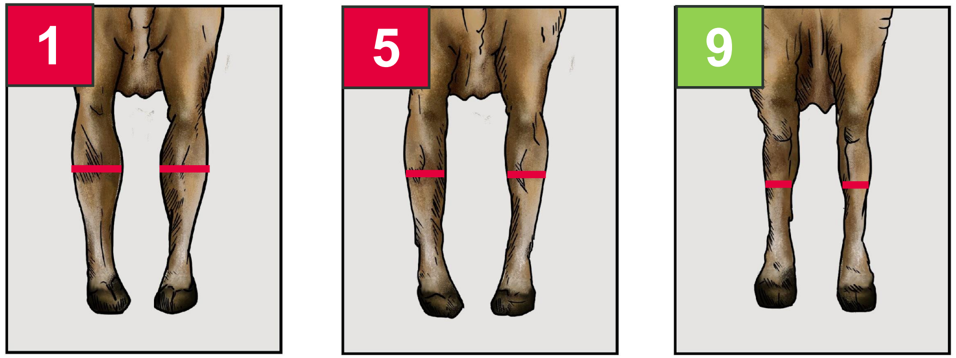
Foot angle (FAN)

Score: 1 to 9



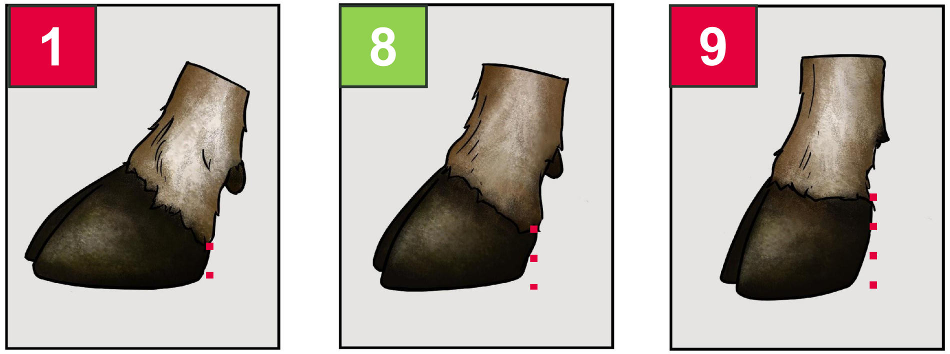
Hock quality (HQU)

Score: 1 to 9



Heel depth (HDE)

score: 1 to 9



Overview: Genotype and Phenotype

Number	Population	
	HO	BS
Phenotype records	1,141,329	1,132,160
Animal genotyped	87,806	52,202
Animal with genotype and phenotype	21,353	15,724
whole genome sequenced level imputed SNV panel	37,471,032	26,455,603
High density SNV panel	777,962	777,962

Estimated h^2

Breed	Trait	Raw phenotype		Corrected phenotype		h^2 (SE)
		Mean (SD)	Min–Max	Mean (SD)	Min–Max	
HO	BST	5.71 (1.26)	1–9	0.00 (1.14)	-4.82–3.84	**0.28** (0.01)
	HDE	35.58 (5.20)	10–60*	0.00 (4.76)	-25.06–25.57	**0.13** (0.008)
	FAN	5.46 (1.18)	1–9	0.00 (1.10)	-4.98–4.38	**0.14** (0.008)
	LOC	5.62 (1.26)	1–9	0.00 (1.14)	-4.88–3.95	**0.10** (0.007)
	RLR	5.73 (1.39)	1–9	0.00 (1.25)	-5.05–3.93	**0.21** (0.009)
	RLS	5.12 (1.12)	1–9	0.00 (1.06)	-4.50–3.82	**0.24** (0.009)
BS	HDE	5.40 (1.08)	1–9	0.00 (0.95)	-4.18–3.97	**0.10** (0.009)
	FAN	5.34 (1.20)	1–9	0.00 (1.10)	-5.29–4.11	**0.14** (0.01)
	HQU	5.40 (1.87)	1–9	0.00 (1.71)	-5.31–5.57	**0.26** (0.01)
	RLV	4.86 (1.12)	1–9	0.00 (1.04)	-4.15–4.61	**0.21** (0.01)

SD = standard deviation, Min = minimum, Max = maximum, h^2 = additive heritability, SE = standard error
BST = bone structure, DHE = heel depth, FAN = foot angle, LOC = locomotion, RLR = rear leg rear view, RLS = rear leg set, HQU = hock quality, RLV = rear leg side view
HO = Holstein, BS = Brown Swiss
*measured in mm

Suggestive QTL

Threshold:
P ≤ $1x10^{-6}$

- HD pannel GWAS: MLMA using GCTA
- Additive, Dominant and Recessive GWAS

Number	Population	
	HO	BS
Phenotype records	1,141,329	1,132,160
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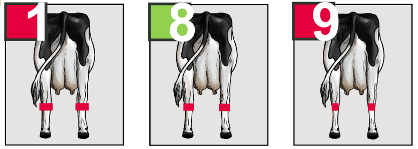
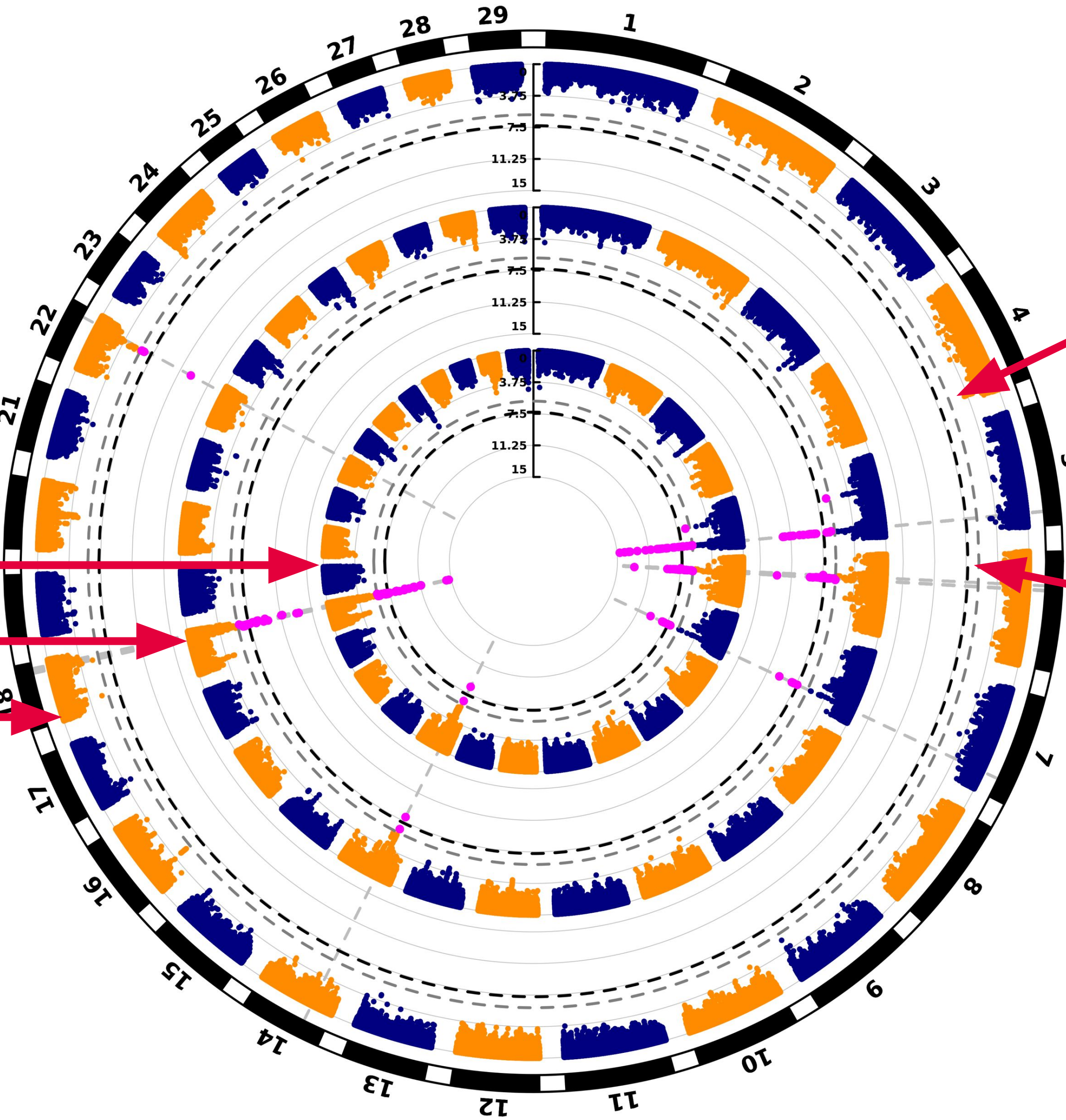
Example: GWAS results for BST in HO





Additive

Dominant

Recessive

Suggestive threshold:
P ≤ $1x10^{-6}$

Significant threshold:
P ≤ $5x10^{-8}$

45 suggestive QTL

Breed	**Trait**	**Additive**	**Non-additive**	
			Dominant	**Recessive**
HO	BST	5	5	1
	FAN	1	2	1
	HDE	1	1	0
	LOC	3	1	1
	RLR	0	0	1
	RLS	2	1	1
BS	HDE	3	2	1
	FAN	0	0	0
	HQU	5	3	0
	RLV	2	1	1
Total		**22**	**16**	**7**

Significant QTL

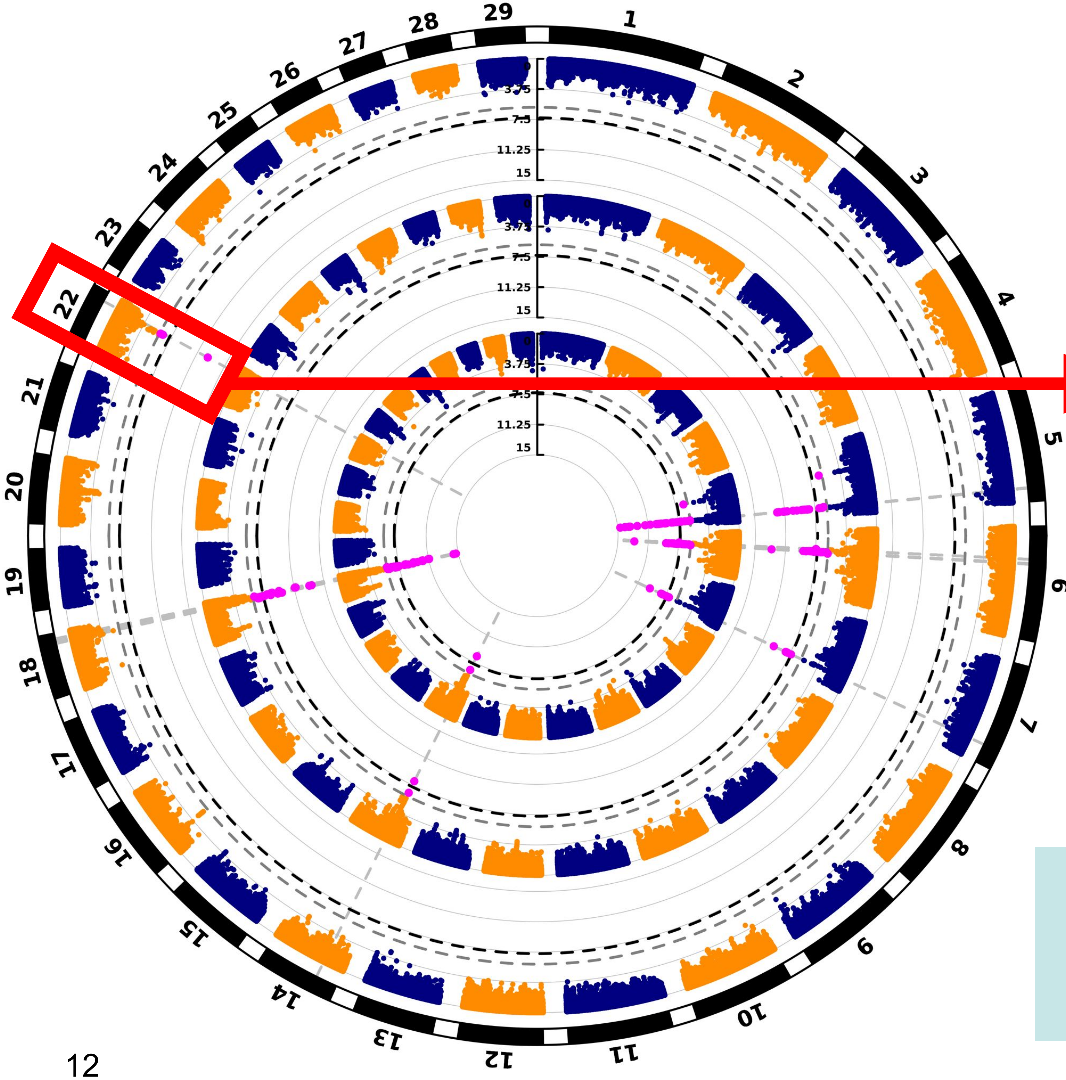
Threshold:
P ≤ 5x10^{-8}

- Whole genome sequencing GWAS: MLMA using GCTA
- Only on chromosome harboring suggestive regions

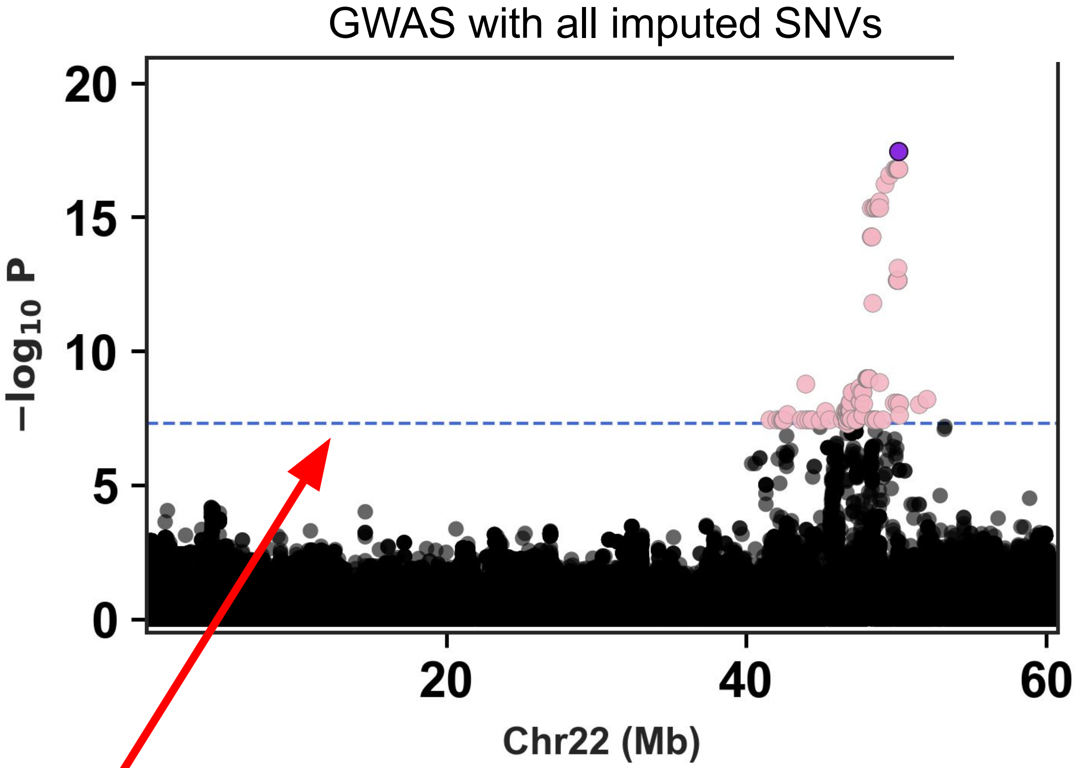
Number	**Population**	
	HO	**BS**
Phenotype records	1,141,329	1,132,160
Animal genotyped	87,806	52,202
Animal with genotype and phenotype	21,353	15,724
whole genome sequenced level imputed SNV panel	37,471,032	26,455,603
High density SNV panel	777,962	777,962

Example: WGS GWAS results for BST in HO

GWAS with HD panel

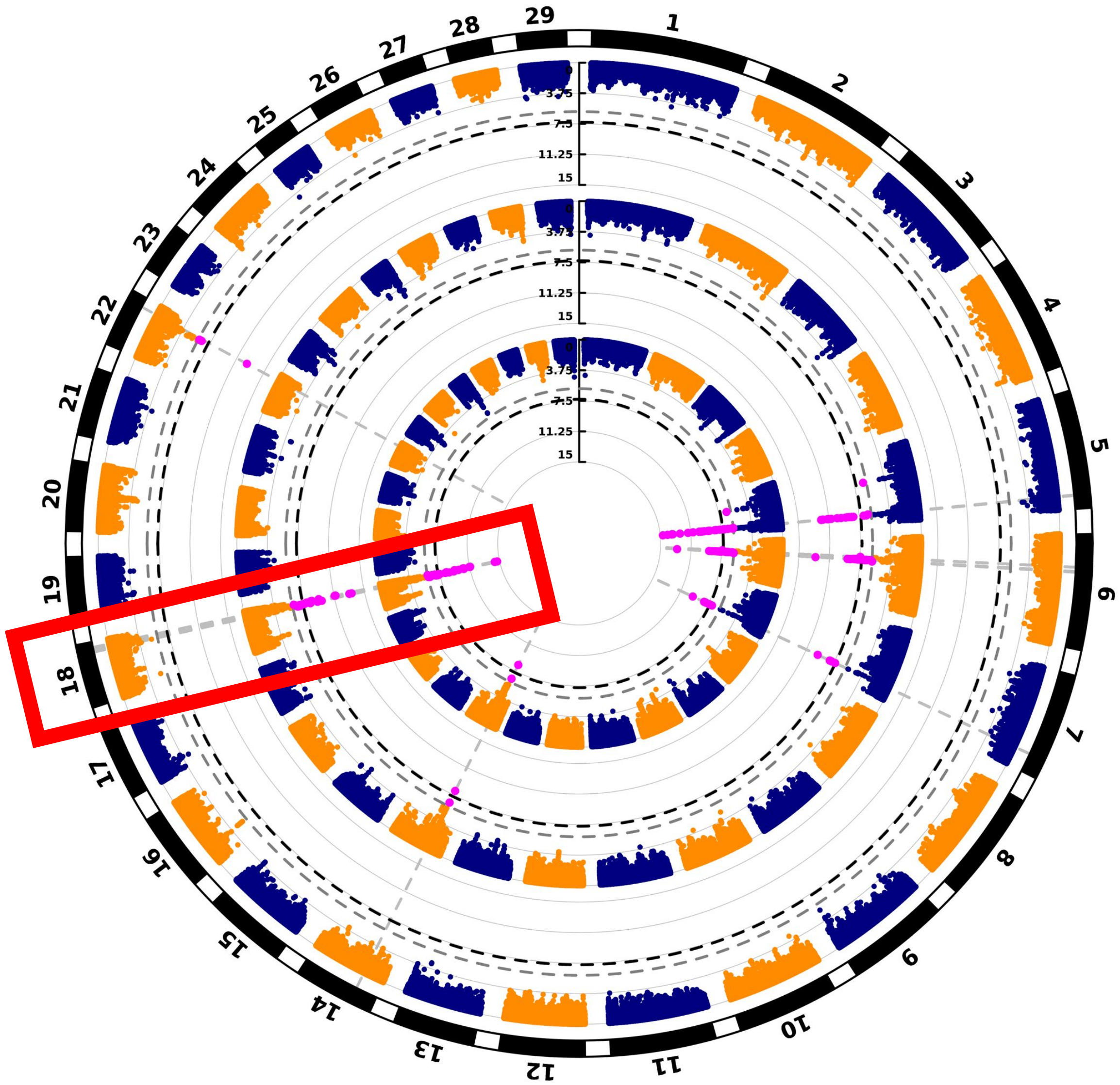


GWAS with all imputed SNVs



Significative threshold:
$P \leq 5 \times 10^{-8}$

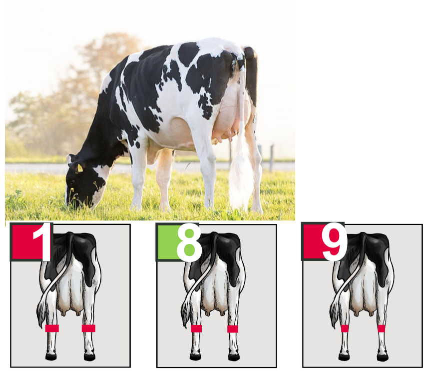
Overlapping of additive and non-additive models

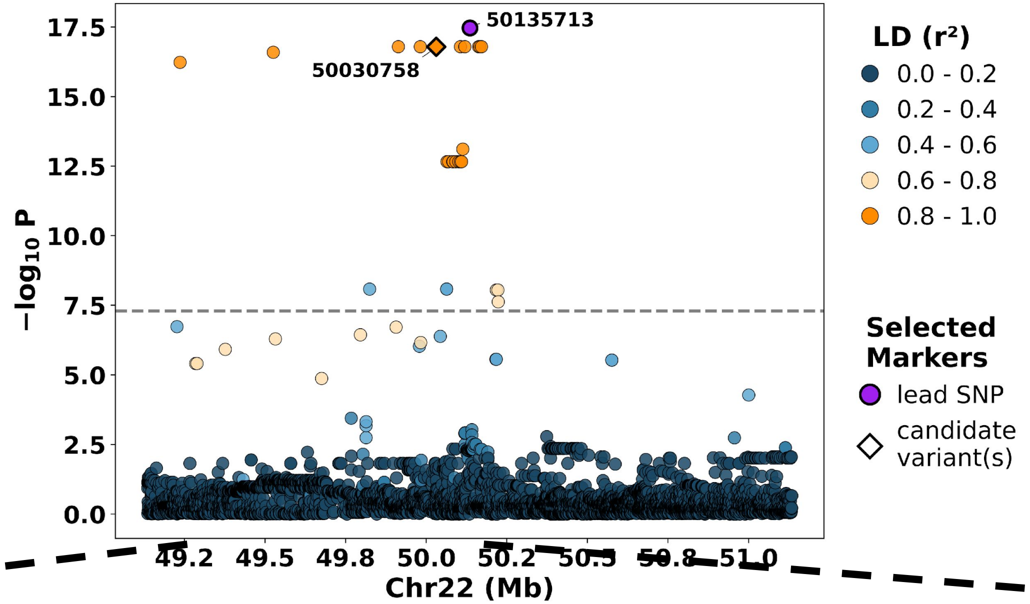


15 significant QTL

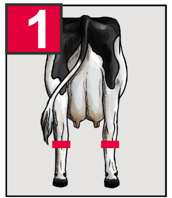
Breed	Trait	Additive	Non-additive	
			Dominant	Recessive
HO	BST	5	0	1
	FAN	0	0	0
	HDE	0	0	0
	LOC	2	0	0
	RLR	0	0	0
	RLS	0	0	0
BS	HDE	2	0	0
	FAN	0	0	0
	HQU	4	1	0
	RLV	0	0	0
Total		**13**	**1**	**1**

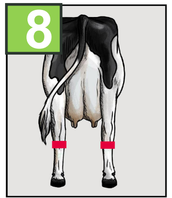
Example: recessive candidate variants for BST in HO

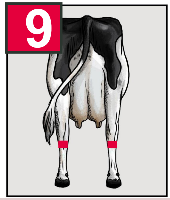




17 candidate variants



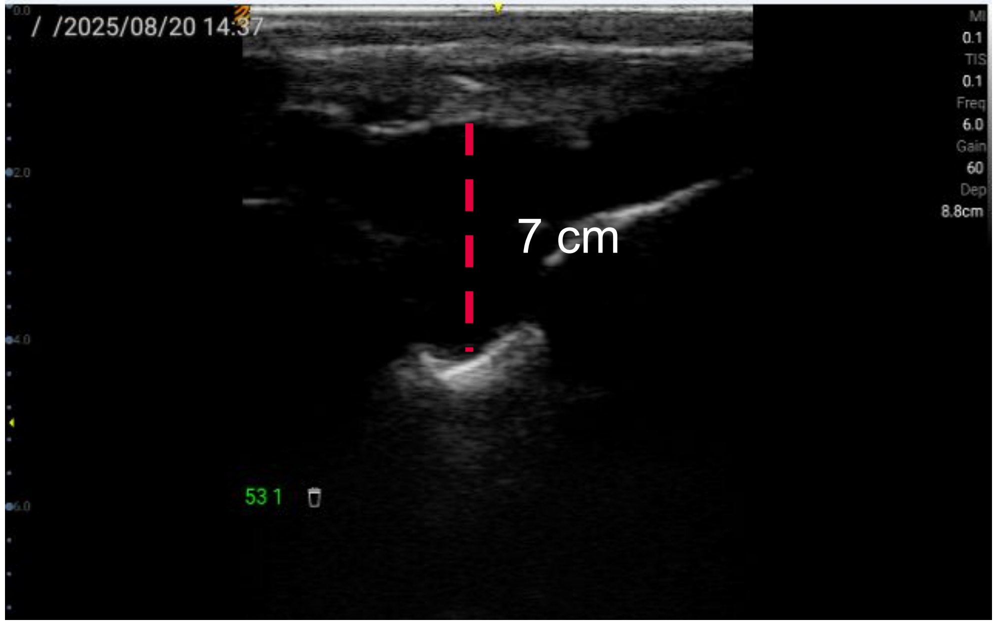




Breed	Trait	Model	Chr	Position (bp)	MAF	R^2	Effect size	SE	p-value	Gene	Variant type
HO	**BST**	Recessive	22	50,030,758	0.043	0.991	-1.594	0.187	1.62×10^{-17}	***HYAL1***	**Stop gained**
	LOC			108,760,988	0.31	1	0.089	0.015	5.42×10^{-9}	*CACNA1C*	Intronic
				44,828,627	0.343	0.944	0.096	0.012	7.31×10^{-15}	***GCC2***	**Missense**
BS	**HD**			61,320,050	0.117	0.995	-0.115	0.020	1.99×10^{-8}	*TPX2*	Synonymous
				1,171,241	0.453	0.951	-0.091	0.015	1.55×10^{-9}	*TELO2*	Upstream
				1,340,769	0.45	0.908	-0.097	0.015	1.55×10^{-10}	*MAPK8IP3*	Intronic
				1,690,471	0.464	0.959	-0.098	0.015	1.15×10^{-10}	*TRAF7*	Upstream
				7	0.466	0.945	-0.096	0.015	2.04×10^{-10}	*ABCA3*	Upstream
	HQU	Additive		45	0.348	0.919	-0.171	0.033	3.12×10^{-7}	*HDAC4*	Intronic
				92	0.348	0.975	-0.181	0.033	5.38×10^{-8}	*NDUFA10*	Intronic
				9	0.456	0.794	0.175	0.032	6.7×10^{-8}	***RIPOR1***	**Frameshift**
			25	1,148,147	0.295	0.978	0.174	0.033	9.85×10^{-8}	*CLCN7*	Synonymous
			26	22,262,227	0.272	0.845	-0.191	0.035	5.76×10^{-8}	*BTRC*	Intronic
				22,433,281	0.273	0.851	-0.194	0.035	3.81×10^{-8}	*FBXW4*	Intronic
				22,503,920	0.273	0.852	-0.2	0.035	1.44×10^{-8}	***FGF8***	**Missense**
				23,248,278	0.303	1	-0.2	0.034	3.24×10^{-9}	*SUFU*	Downstream
		Dominant	6	88,208,422	0.154	1	-0.297	0.045	5.42×10^{-11}	*ANKRD17*	Intronic

Polysynovitis in *HYAL1* homozygous Swiss HO cattle

- **On-farm Examination** of 7 HO females
  - Three heifers 7 mo. old
  - Four cows 3.5 y. old
- **Clinical findings**
  - hock joints enlargement
  - Lameness in one cow (score 3/5)
- **Ultrasonography**
  - Affected joints with effusion
- **Clinical Diagnosis**
  - **Polysynovitis**

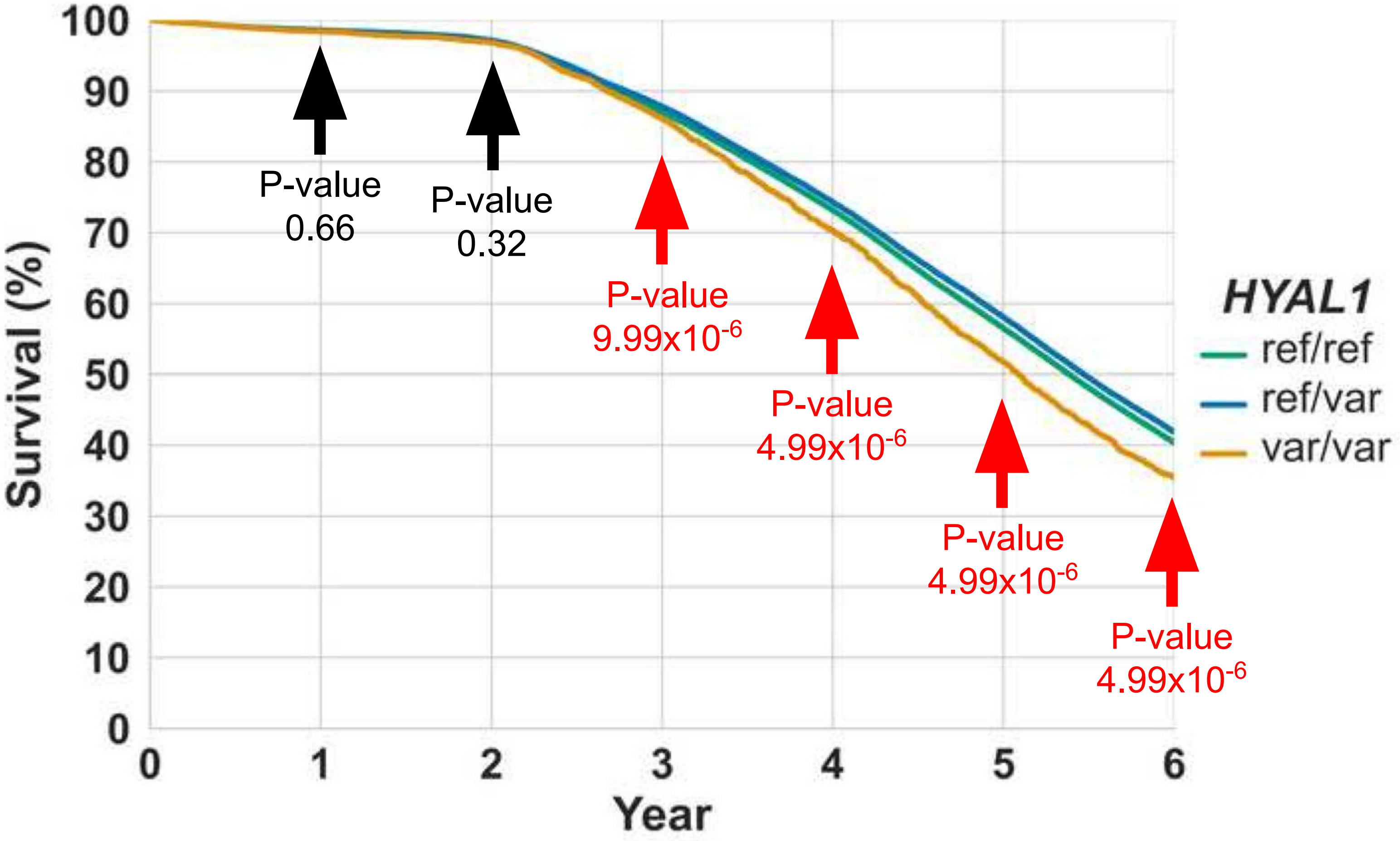


HYAL1 as functional candidate gene

- Function
  - Produces hyaluronidase-1, an **enzyme that breaks down hyaluronic acid**
- Reported effect in humans
  - Mucopolysaccharidosis Type IX in humans
    - soft tissue, joint swelling, cartilage abnormalities, and reduced bone quality
- Predict effect on cattle
  - May disrupt hyaluronic acid breakdown
  - leading to weaker cartilage, altered hoof/bone structure and accumulation of hyaluronic acid

Imundo et al. (2011)

Recessive *HYAL1* nonsense variant affecting longevity in French HO cattle



P-value 0.66

P-value 0.32

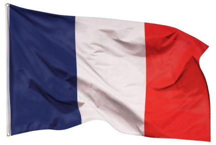
P-value 9.99×10^{-6}

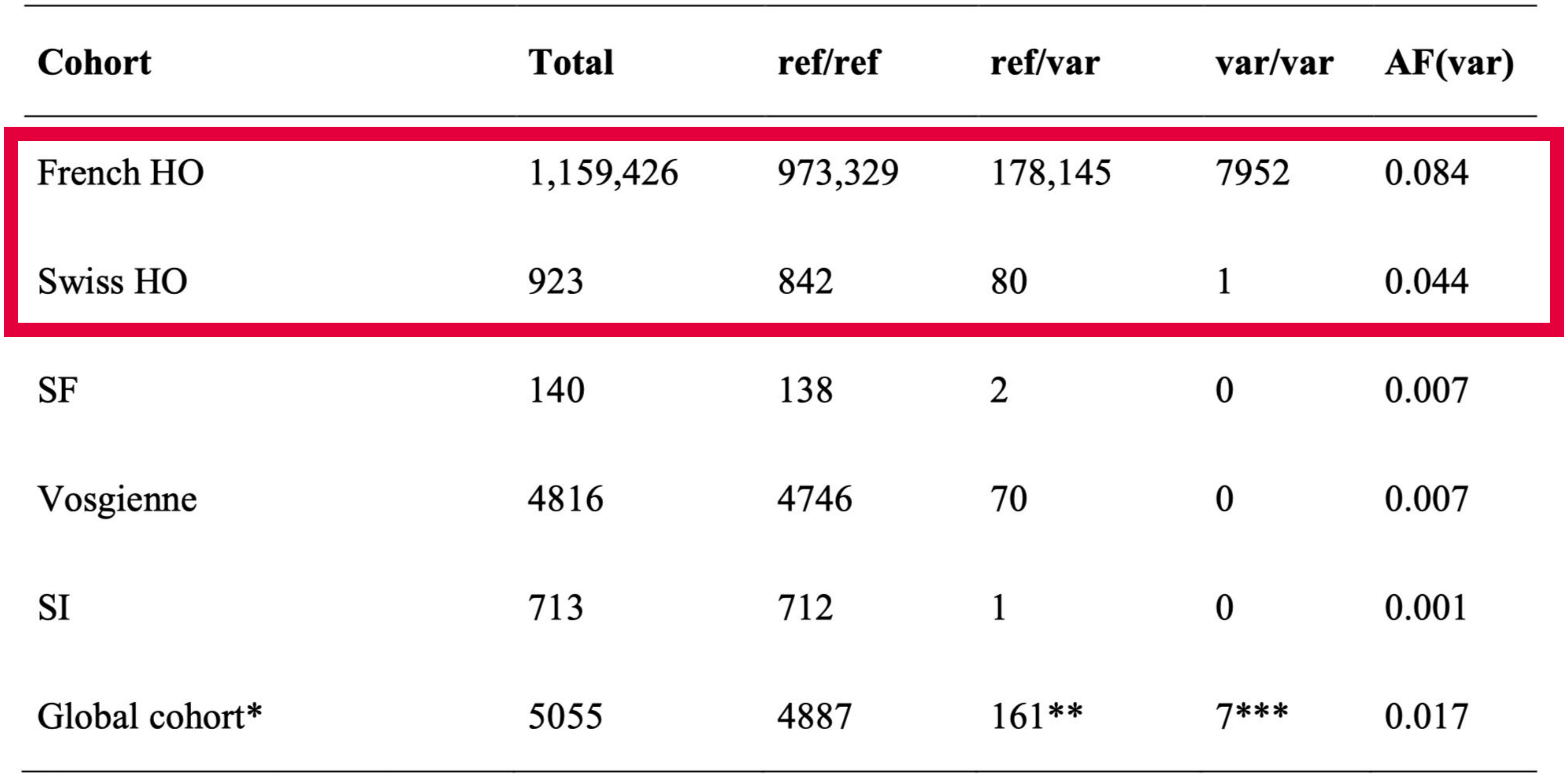
P-value 4.99×10^{-6}

P-value 4.99×10^{-6}

P-value 4.99×10^{-6}

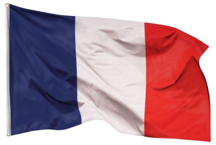
The *HYAL1* variant segregates exclusively in HO

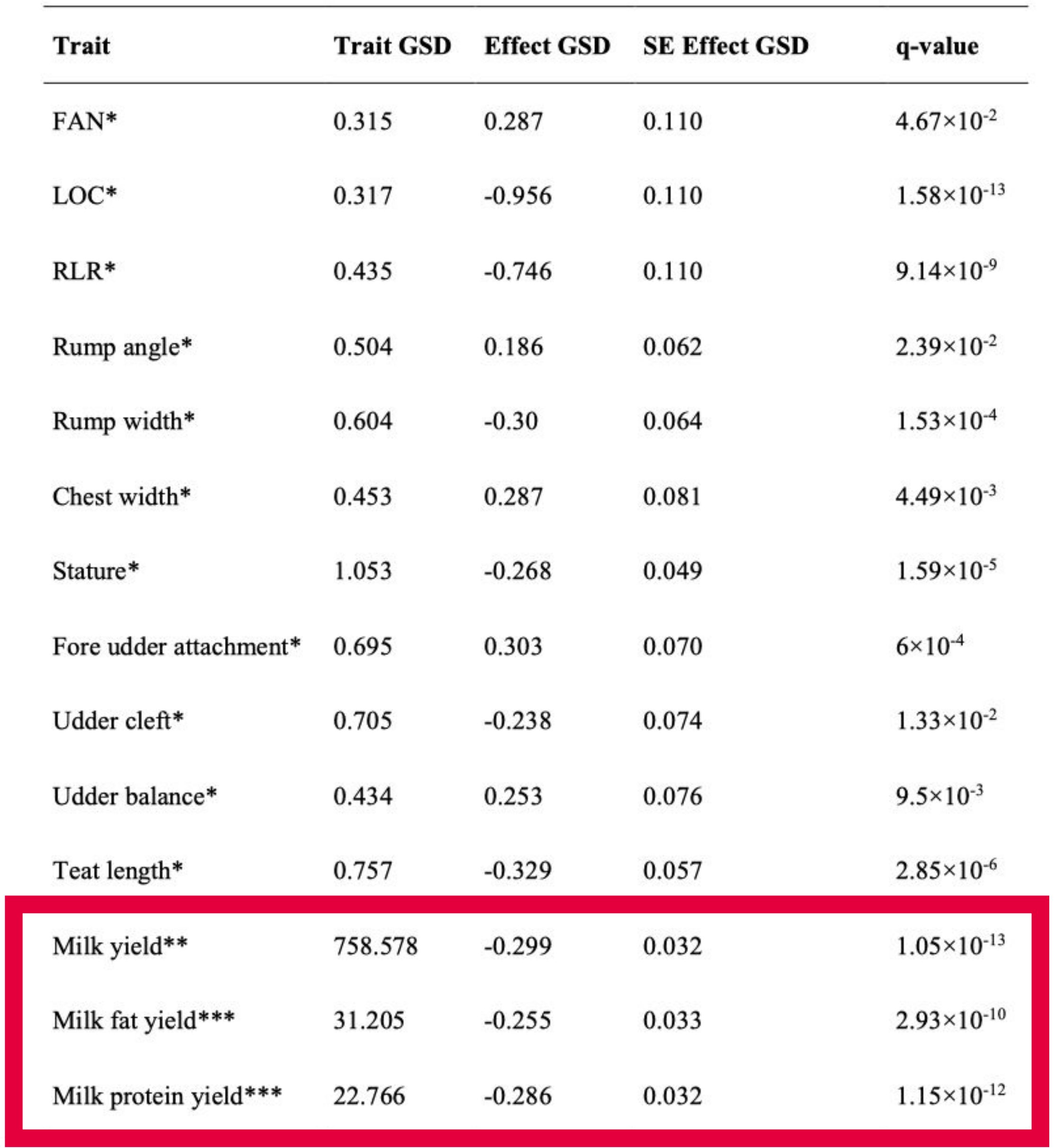




Cohort	Total	ref/ref	ref/var	var/var	AF(var)
French HO	1,159,426	973,329	178,145	7952	0.084
Swiss HO	923	842	80	1	0.044
SF	140	138	2	0	0.007
Vosgienne	4816	4746	70	0	0.007
SI	713	712	1	0	0.001
Global cohort*	5055	4887	161**	7***	0.017

Pleiotropic effects of the *HYAL1* variant





Trait	Trait GSD	Effect GSD	SE Effect GSD	q-value
FAN*	0.315	0.287	0.110	4.67×10^{-2}
LOC*	0.317	-0.956	0.110	1.58×10^{-13}
RLR*	0.435	-0.746	0.110	9.14×10^{-9}
Rump angle*	0.504	0.186	0.062	2.39×10^{-2}
Rump width*	0.604	-0.30	0.064	1.53×10^{-4}
Chest width*	0.453	0.287	0.081	4.49×10^{-3}
Stature*	1.053	-0.268	0.049	1.59×10^{-5}
Fore udder attachment*	0.695	0.303	0.070	6×10^{-4}
Udder cleft*	0.705	-0.238	0.074	1.33×10^{-2}
Udder balance*	0.434	0.253	0.076	9.5×10^{-3}
Teat length*	0.757	-0.329	0.057	2.85×10^{-6}
Milk yield**	758.578	-0.299	0.032	1.05×10^{-13}
Milk fat yield***	31.205	-0.255	0.033	2.93×10^{-10}
Milk protein yield***	22.766	-0.286	0.032	1.15×10^{-12}

Take home message

- First high-resolution analysis
- Traits are polygenic, driven by many small-effect loci.
- Non-additive GWAS models reveal important large-effect recessive variant (*HYAL1*)
  - Monitoring deleterious alleles in breeding populations
  - Improving future genomic evalutions
- **Paper submitted to Genetics Selection Evolution (GSE)**

Thank you !

Interfaculty Bioinformatics Unit

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